

**Physicochemical properties of fermented liquid potato hash diet treated with
or without exogenous enzymes and their effects on feed intake, growth
performance and carcass characteristics of growing Large White x Landrace
crossbred pigs**

By

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Declaration

I, Ronald Sylvester Thomas, declare that this thesis which I have compiled and submitted to the University of KwaZulu-Natal for the Doctoral degree, represents my own work and has never been submitted to any other tertiary institution for any degree.

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LIST OF ACRONYMS AND ABBREVIATIONS

ABBREVIATIONS	DESCRIPTION
%	percent
°C	degrees Celsius
ADF	Acid detergent fibre
ADFI	Average daily feed intake
ADG	Average daily gain
ANOVA	Analysis of Variance
ARC	Agricultural Research Council
AA	Acetic acid
FLPH	Fermented liquid potato hash
BW	body weight
Cfu	colony forming unit
Cont	control
CP	crude protein
DE	digestible energy
DM	dry matter
DMI	dry matter intake
EE	ether extract
FCR	feed conversion ratio

FW	final weights
g	gram
GE	gross energy
h	hour
HCl	hydrochloric acid
HClO ₄	perchloric acid
IW	initial weights
kg	kilogram
L	litre
LA	lactic acid
LAB	lactic acid bacteria
NDF	neutral detergent fibre
SEM	standard error of means
SOC	soya oil cake
Supp	pig supplement
Trt	treatment
VFA	volatile fatty acid
Vs	versus
WSC	water soluble carbohydrate
Wt	weight

Abstract

The main objective of the study was to assess fermented liquid potato hash diet as an alternative feed for pig farmers. In Experiment 1, the physicochemical properties and nutritive value of varying levels of liquid fermented potato hash with or without exogenous enzymes were determined. Different inclusion levels of potato hash were mixed with water at a ratio of 1:2 to ferment for 8 hr. An exogenous xylanase enzyme (Natugrain TS L®) was added at 560 TXU (thermostable xylanase units) and 250 TGU (thermostable glucanase units) per kg feed before fermenting diets. A back-slop fermentation process was followed. Diets were: CON (control diet that was not fermented and without potato hash), LFC (fermented control diet), LLPH (diet containing 200 g potato hash.kg⁻¹ diet, as fed), HLFPH (diet containing 400 g potato hash.kg⁻¹ diet, as fed), LFCE (fermented control diet treated with an exogenous xylanase enzyme (Natugrain TS L®), LLFPHE (diet containing 200 g potato hash.kg⁻¹ diet treated with an exogenous xylanase enzyme (Natugrain TS L®), as fed), HLFPE (diet containing 400 g potato hash.kg⁻¹ diet treated with an exogenous xylanase enzyme (Natugrain TS L®). Fermenting liquid potato hash with exogenous enzymes reduced ($P<0.05$) swelling capacity, acid detergent fibre (ADF) and neutral detergent fibre (NDF), thus making more sugar available for fermentation and increased ($P<0.05$) dry matter, crude protein and water holding capacity compared with fermenting liquid potato hash without enzyme.

In Experiment 2, the microbial characteristics of fermented liquid potato hash diet treated with or without enzyme were determined. Fermented liquid potato hash diets with exogenous enzymes rapidly reduced ($P<0.05$) pH and increased lactic acid (LA) content thus preserving the liquid fermented diets compared to other treatments. The

number of lactic acid bacteria in the LFC+E, LLFPH+E and HLFPH+E was increased ($P < 0.05$) compared to LFC, LLFPH and HLFPH. Enterobacteriaceae bacteria counts in the LFC+E, LLFPH+E and HLFPH+E was decreased ($P > 0.05$) compared to LFC, LLFPH and HLFPH.

In Experiment 3, the impact of non-fermented feed and varying levels of fermented liquid potato hash feed treated with or without exogenous enzyme on feed intake and growth performance of growing Large White × Landrace crossbred pigs were assessed. Forty-two crossbred pigs (Large White × Landrace) aged 55 days with average weight of 25.5 ± 3 kg were fed *ad libitum* diets containing varying levels of liquid fermented potato hash with or without exogenous enzymes. Diets were formulated to provide 14 MJ/kg digestible energy (DE), 180 g crude protein (CP)/kg and 11.6 g lysine /kg. Diets were the control, LFC, LFC+E, LLFPH+E, LLFPH, HLFPH+E and HLFPH diets. The LLFPH+E had greater ($P < 0.05$) final body weight (FW), average daily feed intake (ADFI), dry matter intake (DMI), average daily gain (ADG) and feed conversion ratio (FCR) than the LLFPH, HLFPH, LFC. There was no difference ($P > 0.05$) between LLFPH+E and control diets. The fermented liquid diets treated with enzyme improved ($P < 0.05$) feed intake of grower pigs.

In Experiment 4, carcass traits of cross-breed (Landrace × Large White) pigs fed on varying levels of liquid potato hash based diets fermented with or without exogenous enzyme were assessed. Forty-two grower pigs, (aged 130 days at an average live weight of 60 ± 4 kg) were slaughtered and carcass traits were assessed. There were no differences ($P > 0.05$) in warm, cold carcass weights dressing percentage and carcass length between diets containing varying levels of liquid fermented potato with

or without enzyme. The LLFPH diets with or without enzyme had a higher ($P < 0.05$) drip loss percentage, eye muscle area and lower back-fat thickness and shoulder fat measurements than control, LFC and HLFP with or without enzyme. Therefore, it was concluded that it is economically feasible to produce liquid feed using potato hash and feed grower pigs. Pig performance was comparable to that of control diet. Fermented liquid potato hash that was treated with or without enzyme at low inclusion levels can, therefore, be included in the diet of growing pigs.

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Chapter 1: General Introduction

1.1 Introduction

Inadequate nutrition is one of the major causes for the low productivity of small-scale pig production systems (Henning, 1998). One of the major challenges facing the pig industry is the increased feed costs driven by the high demand for cereals. High feed costs and competition with humans for feed items suggest strongly that alternative energy sources such as food by-products that are normally wasted or thrown away be used partially or totally to replace cereals in livestock diets to reduce and enhance cheaper meat production (Nkosi *et al.*, 2010). Consequently, the use of by-products from food processing industry can be a less expensive source of nutrients suitable for the pig industry. Furthermore, using by-products for pig feeding can be an alternative for the food industry to diminish waste discharges and to reduce waste management costs and environmental pollution.

The process by which by-products are produced increases the concentration of the fibre fraction. To use these ingredients, the animal feed industry has to characterize them accurately to avoid compromising animal performance and to estimate the value of the by-products in least cost formulations. In recent years the use of fibrous by-products has significantly increased in pig diets resulting in increased dietary fibre content (Guillon and Champ, 2000; Elleuch *et al.*, 2011).

Fibrous ingredients are generally bulky and their inclusion reduces nutrient densities of feeds, prompting the pig to increase feed intake (Chaplin, 2003; Anguita *et al.*, 2006). The gut capacity is reached when feed intake of a pig that has not attained its

desired nutrient intake becomes constant or decreases (Takahashi *et al.*, 2009). Finishing pigs are expected to have developed the capacity to ferment and utilise fibrous feeds (Kanengoni *et al.*, 2004). Little has been done to determine the maximum inclusion levels of different dietary fibre sources to allow consumption of sufficient nutrients for potential growth in pigs.

The amount of feed that can be consumed by a pig is undoubtedly determined by physicochemical measures of the feed and the gut capacity of the pig (Canibe and Bach Knudsen, 2002). Physicochemical measurements are the chemical and physical properties of a feed that determine the amount of space it is required to occupy. To date, little effort has been made to determine the influence of physicochemical measurements of fibrous agro by-products in growing pigs. In pigs, the fundamental determinant of performance is feed intake. Prediction of the effects that physicochemical measures of bulkiness might have on the amount of feed consumed is, therefore, crucial (Montagne *et al.*, 2003). The bulkiness of commonly used non-fibrous ingredients is not known. The effectiveness of predicting voluntary feed intake of growing pigs solely depends on understanding relationships among physicochemical properties of a wide range of feed ingredients and their additive effects in pig diets which is usually ignored when describing the nutritional composition of pig feeds (Chaplin, 2003; Anguita *et al.*, 2006). If the physicochemical properties of these bulky feeds are not quantified, their usefulness in feeds may be incorrectly judged and may impose undesirable effects on feed intake, digestibility and, subsequently, growth performance of pigs.

Potential fibrous feed ingredients that can be incorporated in pig diets when predicting feed intake include common, readily available and cheap crop residues and agro-industrial by-products. By-products of industrial potato processing are a potential feed resource which could replace or be included in traditional pig diets. Potato hash (PH), a potato by-product derived from the production of chips and snacks (Schieber *et al.*, 2001), produced at Simba (Pty) Ltd (Isando, Gauteng Province) at the rate of 50 tons per day is one such potential ingredient. This by-product contains a dry matter (DM) of 150 g/kg, 700 g/kg DM of starch, 105 g/kg DM of crude protein, and crude fibre of 58.5 g/kg DM (Nkosi, 2009) and a relatively small amount of yellow maize and fats. The low DM concentration in potato by-products is attributed to the use of large amounts of water in washing and processing. This composition makes potato by-products to be costly when transporting, environmentally difficult to dispose of and limited to be used in pig diets without any treatment.

Treatment methods such as steaming (Radunz *et al.*, 2003), alkali addition (Sauter *et al.*, 1980), freezing (Dolores *et al.*, 2005) and pasteurization (Szasz *et al.*, 2005) have been tried with success in preparing potato by-products for livestock feeding. There are, however, challenges involved in the afore-mentioned methods such as the high costs associated with specialized machinery requirements, which may not be reasonable and sustainable under commercial pig production systems. Although drying of potato by-products reduces the cost of transportation, the selling price may become higher (Kajikawa, 1996). Mechanical drying of wet by-products increases the price of the by-products substantially, hence, much of the wet by-products are discarded or freshly fed only on limited periods of time. This may result in a waste of potential feed resources and environmental pollution. Another option may be sun-

drying, which depends on the weather condition (Silayo *et al.*, 2003). Sun-drying may eliminate toxic substances (Gowda *et al.*, 2007). The need to control birds and rodents which may want to feed on the by-product, however, renders this method unattractive. By-products with high moisture content deteriorate or become moldy very quickly (Kajikawa, 1996). Utilizing the wet by-products as liquid fermented feed could be an alternative in pig diets and could reduce cost of feed. Traditional food fermentations can take potentially hazardous raw materials and transform them into products with both improved storage qualities and a reduced risk of causing illness (Adams and Mitchell, 2002). However, the extent to which fermented foods are safe and how fermentation processes should be conducted to achieve a required level of safety are key questions (Adams and Mitchell, 2002). The use of liquid feed in pig nutrition has recently gained interest for several reasons: (1) the political wish of decreasing the use of antibiotics in pig production; (2) the current fluctuations in feed prices, which makes liquid feed, with the possibility of using cheap liquid ingredients, an interesting feeding strategy; (3) the policies aiming at increasing production of biofuels with a corresponding increase in liquid co-products from the bioethanol industry, suitable for liquid feeding; (4) environmental policies aiming at decreasing disposal of products, for example, liquid by-products from the food/ pharmaceutical/ biofuel industry, which can be included in liquid feeding and thereby contribute to avoid wasteful disposal and decrease the environmental burden (Adams and Mitchell, 2002).

Pigs do not produce enzymes that are able to degrade non-starch polysaccharides (NSP) (Jones *et al.*, 2010). These substrates are considered as indigestible in the small intestine but in the large intestine a variable fraction of fibre will be fermented by the microflora to short-chain fatty acids, and thereby serve as a source of energy for

the pig (Graham *et al.*, 1988). In growing pigs, however, the capacity of the microflora to degrade the NSP is less developed than in older pigs (Graham *et al.*, 1988). Therefore, the lack of enzymatic capacity might be compensated for by supplementation of the diet with exogenous enzymes.

Exogenous enzymes improve nutrient digestion in pigs (Jones *et al.*, 2010; Kerr and Shurson, 2013). Currently there is limited data available on the use of exogenous enzymes to reduce fibre levels in potato hash. Also, there is limited literature on the nutritional composition and effect of liquid fermented potato hash diet on feed intake and growth performance of growing pigs. The lack of such information makes it difficult for stock-feed manufacturers to formulate feeds containing fibrous materials that will allow the optimum utilization of nutrients.

1.2 Justification

The current pressure on grain markets may increasingly force pig producers to substitute the cereal in conventional growing pig diets with cheaper, readily available feed ingredients. Potato hash is a valuable underutilized resource which if exploited as a pig feed ingredient could benefit pig producers. An estimated amount of 50 tons of potato hash is produced per day at Simba (Pty) Ltd (Isando, Gauteng Province) (Nkosi *et al.*, 2015). If it is not consumed in a short period, it gets mouldy and becomes useless as an animal feed.

Each feed ingredient needs to be described in such a way that it is possible to determine its maximum inclusion level in a pig diet. Understanding physicochemical properties of fibrous ingredients assists pig nutritionists to determine the amount of a

given fibre source that can be included in a feed. Investigating the effect of fermented liquid potato hash on physicochemical properties of diets, feed intake, growth performance and carcass characteristics, therefore, assist pig producers to better utilize potato hash. There is need to develop effective technologies that can increase utilisation of potato hash.

Fermented liquid feed is of particular interest as a tool to preserve high moisture by products. Liquid feeding of grower pigs using fermented liquid potato hash has not been adequately studied. Most of the studies on fermented liquid feed are done in Europe, and are based on wheat and barley diets with liquid by-products sources mostly from dairy industries, and results have been variable. South Africa is rich in non-conventional agro-industrial by-products (such as potato hash) and feed companies need to develop strategies to effectively utilize locally available by-products into pig feed, hence reducing environmental pollution and feed costs which the farmers face.

1.3 Objectives

The broad objective of the study was to evaluate liquid fermented potato hash diet as an alternative feedstuff for pig farmers. The specific objectives were to:

1. Characterize physicochemical properties and nutrient composition of varying levels of liquid fermented potato hash diets treated with or without enzymes;
2. Determine the microbial characteristics of varying levels liquid fermented potato hash diet treated with or without enzymes;

3. Compare feed intake and growth performance of growing Large White × Landrace crossbred pigs fed on different inclusion levels of potato hash treated with or without exogenous enzymes; and
4. Determine carcass characteristics of pigs fed on different inclusion levels liquid fermented potato hash feed.

1.4 Hypotheses

The following hypotheses were tested:

- a) Physicochemical properties and nutrient composition of varying levels of liquid fermented potato hash diet are not variable;
- b) Liquid fermented potato hash diets do not have variable microbial characteristics;
- c) Liquid fermented feed do not improves feed intake and utilization of feed by grower pigs; and
- d) Inclusion levels of liquid fermented potato hash feed do not influence carcass characteristics of grower pigs.

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Review of Literature

2.1 Introduction

Liquid feeding systems have been used for many years (Scholten, 2001) to utilize liquid by-products in pig production feeding systems. The use of this technology has, been limited around the world until recently. The increasing popularity of using liquid feeding systems is being driven by the desire to decrease the use of antibiotics in pig production, extremely high prices of conventional dry feeds, a tremendous increase in availability and low cost of liquid by-products from biofuels production, and environmental policies aiming at decreasing disposal of products. For example, liquid by-products from the food industry, and numerous growth performance, health, and animal well-being advantages that liquid feeding systems provide compared to dry feeding systems (Adams and Mitchell, 2002).

Typically, liquid diets contain 20 to 30 % dry matter. In some liquid feeding systems, partial fermentation of ingredients or diets is allowed to occur, resulting in production of organic acids and proliferation of beneficial bacteria, such as *Lactobacillus acidophilus* (de Lange *et al.*, 2006). One of the most important aspects of using liquid fermented feeding successfully is to ensure that the proper ratio of water to dry matter content and frequency of feeding is achieved for the specific production phases where it is used.

2.2 Definition of fermented liquid feed

It is important to define liquid feeding and differentiate it from other feeding systems. Liquid feeding involves the use of a diet prepared either from a mixture of liquid food

industry by-products and conventional dry materials, or from dry raw materials mixed with water. A liquid feed should not be confused with wet/dry feeder systems where water and feed are kept separate up to the point of delivery to the pig (Brooks *et al.*, 2003a).

By definition, fermented liquid feed (FLF) is feed mixed with water, at a ratio of 1:1.5 to 1:4 and fermented for a period long enough to reach steady state conditions (Chae, 2000; Brooks, 2003). It is usually prepared by spontaneous fermentation (spontaneous fermented liquid feed; SFLF) or inclusion of the feed mixture with a culture of lactic acid bacteria (LAB) as inocula (controlled or inoculated fermented liquid feed; IFLF) (Brooks, 2003). If there is almost no time between mixing and feeding or the period for fermentation is too short to reach steady state conditions, the term liquid feed (LF) or non-fermented liquid feed (NFLF) is used (Canibe and Jensen, 2003; Plumed-Ferrer and Von Wright, 2009; Brooks, 2008). Some liquid feed systems allow partial fermentation of ingredients, resulting in the production of organic acids and proliferation of beneficial bacteria, such as *Lactobacillus acidophilus* (Braun and de Lange, 2004; de Lange *et al.*, 2006).

2.3 Benefits of fermented liquid feeding versus dry feeding

There are many advantages of using fermented liquid feeding systems compared to dry feeding in pig production. These include improved nutrient utilization, flexibility and control of feeding programs, utilization of inexpensive liquid by-products, reduced environmental impact, and improved animal performance (Russell *et al.*, 1996; Jensen and Mikkelsen, 1998; Brooks *et al.*, 2001; Lawlor *et al.*, 2002; Canibe and Jensen, 2003). The other beneficial effects of feeding pigs with liquid feed or fermented liquid

feed (FLF) are related to feeding behaviour and gastrointestinal health (Lawlor *et al.*, 2002). Pigs fed fermented liquid feed do not have to learn separate patterns of feeding and drinking behaviour, and this can help in preventing dehydration and a drastic drop of feed intake. Avoiding a drastic decrease in feed and water intake after weaning is expected to ameliorate the post-weaning lag period in weaner pigs. The use of FLF/liquid feed, in contrast to dry feed, is considered a possible feeding strategy to keep a high and regular feed and water intake of pigs. Growth performance of pigs fed with FLF compared to those fed with non-FLF or dry feed is variable (Jensen and Mikkelsen, 1998; Canibe and Jensen, 2003; Brooks, 2008). Fermented liquid feeding has no particular benefits in growth performance (Lawlor *et al.*, 2002; Pedersen *et al.*, 2005; de Lange *et al.*, 2006). Another suggested benefit associated with feeding diets in a fermented liquid form is that pigs are provided with water and feed simultaneously (Russell *et al.*, 1996; Brooks *et al.*, 2001; Brooks and Tsourgiannis, 2003; Brooks *et al.*, 2003a; Brooks, 2008). Scott *et al.* (2007) reported that fermented liquid feeding of growing-finishing pigs lowered gastric ulcer scores at slaughter compared to dry pellet feeding. Other authors also reported that ileal, cecal and colon digesta samples from fermented liquid fed pigs had lower coliform ratios, indicating improved gut health (Hillman *et al.*, 2004; MLC, 2004).

Providing the feed in a liquid form most often results in a higher feed intake after weaning (Russell *et al.*, 1996). The optimal dry matter content of fermented liquid feed varies with the pig's stage of growth, feed composition, and environmental conditions. Feeding liquid diets containing fermented ingredients have resulted in improved growth performance, and reduced mortality and morbidity in nursery and growing-finishing pigs (Geary *et al.*, 1996; 1999; Scholten *et al.*, 1999). These benefits appear

to be due to enhanced nutrient availability, and reduced growth and shedding of pathogenic bacteria such as *Yersinia*, *Salmonella*, and *E. coli* due to low pH (Geary *et al.*, 1996; 1999; van Winsen *et al.*, 2001; Demeckova *et al.*, 2001). Furthermore, pepsin activity is increased due to lower pH resulting in improved protein digestion (Scholten *et al.*, 1999). The presence of lactic acid bacteria and organic acids (lactic acid and butyric acid) in fermented liquid feeds may also have positive effects on digestive and immune functions (Simon *et al.*, 2003).

The influence of FLF feeding compared to dry feed or non-FLF on gastrointestinal health has been reported. These include reduction in the number of several pathogens in the gastrointestinal-tract (GI-tract) of piglets, growing pigs and sows (Mikkelsen and Jensen, 2000; Moran, 2001; van Winsen *et al.*, 2001b; Demeckova *et al.*, 2002; van Winsen *et al.*, 2002; Canibe and Jensen, 2003; Højberg *et al.*, 2003; Hong *et al.*, 2009); reduction of the period with faecal pathogen excretion and lower incidence of clinical disease in challenged animals; and lower risk of pathogen shedding (Dahl, 1997; van der Wolf *et al.*, 1999, 2001a,b; Pedersen *et al.*, 2002; Lindecrona *et al.*, 2003; Boesen *et al.*, 2004; Bahnson *et al.*, 2006; Farzan *et al.*, 2006; Poljak *et al.*, 2008). Benefits of FLF feeding over dry feeding have been long been established. Both Lawlor *et al.* (2002) and Choct *et al.* (2004b) concluded that wet feeding was superior to dry feeding in most studies with the exception of just a few which found no difference in performance. Other studies reported from 1972 to 2004, which are summarized in Table 2.1 showed the advantages of FLF feeding over dry feeding in ADG and F: G ration.

Table 2.1: Comparison of liquid feeding versus dry feeding for growth performance and carcass quality in pigs

Average daily gain	Feed-gain ratio	Carcass quality	Reference
+	+	0	Kneale, 1971
+	+	0	Smith, 1976
+	0	NF	Bradue and Newport, 1977
+	NF	NF	Lecce <i>et al.</i> , 1979
0	-	NF	Kornegay <i>et al.</i> , 1981
+	+	-	Patterson, 1989
+	+	NF	Patridge <i>et al.</i> , 1992
+	+	NF	Upton, 1993
+	+	NF	Pluske <i>et al.</i> , 1996a
+	+	NF	Pluske <i>et al.</i> , 1996b
+	+	NF	Kim <i>et al.</i> , 2001
0	0	NF	Lawlor <i>et al.</i> , 2002
+	0	NF	Canibe and Jensen, 2003
+	-	NF	Choct <i>et al.</i> , 2004b

Note + Liquid is better ; - dry is better ; 0 No difference ; NF No information

2.3.1 Important physicochemical aspects of feed influenced by liquid feeding

Water holding capacity (WHC), bulk density, neutral detergent fibre, crude fibre, viscosity, solubility and swelling capacity are the major physicochemical aspects used to describe feed bulk. They determine the physiological effects and fate of dietary fibre along the GIT and are important for effective digestion (Guillon and Champ, 2000).

2.3.1.1 Water holding capacity

Water holding capacity describes the ability of fibre to trap or immobilise water within its matrix, swell and form gels with high water contents (Kyriazakis and Emmans, 1995). As the WHC of the feed increases, more space will be required in the gut, thereby reducing feed intake (Tsaras *et al.*, 1998). Technically, WHC describes the amount of water that can be held or taken up by a known amount of fibre under known conditions (Guillon and Champ, 2000; Elleuch *et al.*, 2011). The term WHC is often used in relation to swelling capacity and viscosity of the material under investigation (Elhardallou and Walker, 1993; Takahashi *et al.*, 2009).

Fibre polymers bind water at differing strengths and in different quantities. Water in digesta can be held by dietary particles or remain unbound as either trapped or free water (Chaplin, 2003; Anguita *et al.*, 2006). Water holding capacity of fibre matrix along the gut depends on the conditions of each particular GIT segment (Canibe and Bach Knudsen, 2002). It is important that when an investigation on physicochemical implications of fibre source and inclusion level is conducted, samples for measurements be taken from representable sites of the GIT. Common methods for measuring WHC include filtration, centrifugation and use of dialysis bags (Elhardallou and Walker, 1993; Kyriazakis and Emmans, 1995).

The WHC of fibre sources differ according to the variation in the monomeric composition of these fibrous materials and structural arrangement of their molecules. For example, Serena and Bach-Knudsen (2007) reported that WHC is greater in pectin, potato pulp, and sugar beet pulp than in seed residues, pea hulls, and intermediate in wheat and barley. The differences noticed in WHC of fibrous ingredients warrants prediction of gut capacity to be done using a wide variety of fibrous sources.

Water holding capacity interacts with other physical properties of fibres. For example, as the ability of the fibre source to immobilise water increases, the bulk density of the fibre matrix also increases (Kyriazakis and Emmans, 1995). The relationship between WHC and other chemical properties of fibres can be explained by the prevalence of hydrogen bonds within the fibre matrix and the extent with which these bonds are exposed as possible binding sites (hydroxyl groups) with water molecules (Oakenfull, 2001). As a result, processing of feed ingredients, such as grinding or chopping may influence exposure of hydroxyl groups, and subsequently, the WHC of the feed. The relationship between WHC and feed intake in weaner and finishing pigs is still unclear. Investigating dynamics change in conjunction with other physicochemical properties of these fibrous is critical so that relationships between bulkiness and gut capacity can be accurately predicted.

2.3.1.2. Bulk density

Bulk density is the degree of consistency measured by the quantity of mass per unit volume occupied by the fibrous material (Kyriazakis and Emmans, 1995). Inclusion of levels of fibrous ingredients which are lighter and fluffier than the conventional diet

caused a decrease in the quantity of less bulk material per unit volume that could be consumed implying that more space per unit mass will be required to accommodate the feed in the gut. The bulk density of the digesta is subject to change in different sections of gut depending on the inclusion level of fibre in the diet and rate of water absorption during gut transit. As feed absorbs water in the gut, the volume of the ingesta is increased in such a way that the bulk density increases. Information on how the bulk density of feed constrains feed intake in pigs is undermined. There is need, therefore, to predict the gut capacity of pigs using bulk density of fibrous diets formulated from fibre sources with a widespread range of bulky densities.

2.3.1.3 Swelling capacity

Swelling capacity is the volume occupied by a known weight of fibre as it absorbs water within its matrix (Borchani *et al.*, 2011). Feeds that have a high swelling capacity occupy more space in the gut, thereby limiting feed intake. There is little knowledge on the effects of swelling capacity of the feed on feed intake. The swelling ability of feed components during gut transit is likely to be pronounced more in finishing pigs than weaner pigs, thus, intake patterns during these phases may differ. Although the physicochemical properties form part of the dietary factors of feed intake, it is crucial that animal and environmental factors affecting feed intake are understood.

2.3.1.4 Neutral detergent fibre

The NDF is another common measure of fibre content that can be used to predict gut capacity in growing pigs. It measures most of the structural components in plant cells, i.e. lignin, hemicellulose and cellulose (Van Soest *et al.*, 1991). An increase in

inclusion level of fibre in the diet is associated with an increase in NDF content, reduction in nutrient density and bulkiness. Therefore, when growing pigs are given a diet with high NDF, the levels of feed intake will increase to compensate for the reduced content of digestible material, especially in weaner pigs (Bindelle *et al.*, 2008). However, in finishing pigs, due to their ability to digest fibre and elongation of their large intestines and caecum, the anticipated patterns are likely to vary.

2.4 Common by-products used in liquid feeding systems

Liquid feeding for pig diets can use ingredients from a wide range of sources such as food industries, milk processing, and candy, bakery and alcohol production products. Limited nutritional information is available for some products, and there is concern on their variability of contents. Many products also have not been evaluated for pathogens or harmful chemicals and may represent a food safety risk (Brooks *et al.*, 2001). There is wide range of by-products that have been used in pig production in different countries and are potential ingredients for use in fermented liquid feeding system.

2.4.1 Liquid whey

Whey is the by-product from cheese production (Olstorpe *et al.*, 2008). Whey typically has a pH of 5. If it has not been treated with excessive heat, whey contains highly digestible protein and is an excellent, highly digestible energy source for pigs, because it contains approximately 60 % lactose. Due to the decreasing ability of the pig to effectively digest lactose after weaning, digestive upsets can, however, occur in older pigs when it is included in liquid feed or fed at high dietary levels. Additional access of

water should also be provided to avoid salt toxicity due to high salt content (Braun and de Lange, 2004).

2.4.2 Brewer's wet yeast (BWY)

Fresh brewer's yeast is produced by removal of culture from fermenting beer wort by flocculation or sedimentation. After separation of yeast from beer, it will have 30 % DM, after pressing to remove any remaining beer. Brewer's yeast contains active yeast which may cause further fermentation and frothing during storage, the build-up of pressure in liquid feed lines and bloating of pigs. As a result, organic acids should be added to deactivate yeast or heating should be applied to kill live yeast prior to shipping (Ruis, 2003). Fresh brewer's yeast is an excellent source of protein of high biological value and digestibility. It is high in B complex vitamins, and a rich source of enzymes and cofactors that can be fed to pigs to enhance productivity (Kornegay *et al.*, 1995). It is usually added at a rate of between 2 and 5 % in pig diets but can be used to replace up to 80 % of the protein if it is inexpensive. Good growth performance can be achieved when feeding wet brewer's yeast but the response varies with the stage of production when it is fed (Van Heugten *et al.*, 2003; Braun and de Lange, 2004).

2.4.3 Sugar syrup

Sugar syrup contains approximately 65 % DM and is high in energy but essentially low in protein, vitamins, and minerals. High sugar content can cause digestive upsets in pigs and therefore, should be limited to no more than 5 % of the diet (de Lange *et al.*, 2006).

2.4.4 Bakery waste

Bakery waste includes bread, cookies, crackers and other confectionaries and can vary markedly in nutrient content depending on the type of food by-products in the mixture. Bread is high in energy but may require special handling equipment to remove wrappers (Braun and de Lange, 2004). Bread meal should be limited to no more than 30 % of DM intake for pigs (de Lange *et al.*, 2006). Cookies and crackers can contain high amounts of fat and sugars making them excellent energy sources. Depending on the type of bakery product, salt content can be relatively high and should be taken into account when formulating liquid feed supplements. It is usually about 90 % dry matter, 10 % crude protein, less than 1 % crude fibre and between 3 and 5% ash. Bakery waste can replace all grain in the pig diet (de Lange *et al.*, 2006).

2.4.5 Potato waste

Potato by-products from a processing plant are a generic description for a heterogeneous mixture of potato components that varies depending on the nature of the processing method. They may contain varying amounts of inedible spoiled potatoes, chips, peels and fats (Schroeder, 1999). Potato wastes are primarily energy sources, being approximately 13 MJ/kg DM (Rooke *et al.*, 1997) and varying fat concentration from 5 to 10 % (Duynisveld and Charmley, 2003). These wastes are poor in dry matter, protein, mineral and fibre contents. Their crude protein ranges between 4 and 8 % and with a fibre content that ranges between 1.6 and 17.5 %, respectively (Onwubuemeli *et al.*, 1985). Such a variation depends greatly on the processing methods used.

Potato hash has a DM of 150 g/kg, starch of 700 g/kg DM and a relatively small amount of yellow maize and fats (Nkosi *et al.*, 2010). Its moisture content of 85 % is comparable to that of potato pulp, which is 83 % (Okine *et al.*, 2005). The low DM concentration in potato by-products is attributed to the low DM content of potatoes plus the use of large amounts of water in washing and processing, some of which remains in the by-product.

Research trials substituting barley for potato by-products have generally shown modest increases in digestibility (Onwubuemeli *et al.*, 1985). Feeding potato by-products to livestock has been successful in the North West of Pacific in the USA where an estimation of 98 % of cattle are fed (Bradshaw *et al.*, 2002). In Canada, large volumes of potato by-products are produced and used as fertilizers. A growing interest in using these by-products in pig feeding has emerged (Charmley *et al.*, 2006). Higher dietary inclusion levels (>20 %) of potato by-products may depress dry matter intake, and pigs need more time to adapt to the ration.

2.5 Conventional methods used to treat potato by-products for pig feeding

Treatment methods such as steaming (Radunz *et al.*, 2003), alkali addition (Sauter *et al.*, 1980), freezing (Dolores *et al.*, 2005) and pasteurization (Szasz *et al.*, 2005) have been tried with success in preparing potato by-products for livestock feeding. There are, however, challenges such as the cost involved in the afore-mentioned methods due to machinery requirements, which may not be attainable under small-scale systems. Drying of by-products reduces the cost of transportation, although the selling price may become higher (Kajikawa, 1996). Machinery drying of wet by-products increases the price of the by-products substantially, hence, much of the wet by-

products are discarded or fresh fed only on limited periods of time. For most other by-products, however, the price of the dried form does not cover the drying cost, and they may be disposed without having being used as feed, which results in a waste of potential resources and environmental pollution. Another option may be the sun-drying method, which is more weather dependent (Silayo *et al.*, 2003) and may eliminate toxic substances (Gowda *et al.*, 2007). The sun-drying method is, however, sometimes associated with the presence of birds that might feed themselves on the by-product while drying, and may render this method unsuccessful. By-products with high moisture content tend to deteriorate or become mouldy very quickly (Kajikawa, 1996).

In such cases, fermenting can be a way to preserve by-products of such a nature, as evidenced in work done by Brooks (2008), and Plumed-Ferrer and Von Wright (2009). Fermented products generally have a longer shelf life than their original substrate (Adams and Mitchell, 2002). The inhibitory action is also one of the positive things associated with the use of fermented feed in pigs. Fermenting of agro-industry by-products is a simple and appropriate method of conservation which is less weather dependent, and research has shown that it is the most suitable method of conservation for short periods (Brooks, 2003; Brooks *et al.*, 2003a) and for the reduction of pathogenic agents (Martinez-Gamba, 2001).

2.6 Important animal factors influenced by fermented liquid feed

Fermented liquid feed influences the palatability and voluntary feed intake, gastrointestinal health, growth performance and carcass characteristics.

2.6.1 Palatability and voluntary feed intake

One key factor for a profitable pig production is, of course, to maintain a high growth rate and feed intake of the pig. A good health status is important when it comes to feed consumption but the palatability of the diet is crucial. It is therefore very important to have an accurate knowledge of the different dietary factors that can affect the palatability of diets for pigs. Even though the growth-promoting effects of organic acids depend, to a large extent, on how they improve feed intake, there are few studies on the effect they have on the palatability of the feed. It is considered that high dietary levels of certain organic acids can reduce the palatability of the diet substantially and affect the pigs feed intake. In a study when pigs had access to both an acidified (citric and fumaric acid) and non-acidified diet, they consumed significantly more of the non-acidified diet (Partanen and Mroz, 1999).

In general, lactic acid is considered to enhance the palatability of the feed even at higher inclusion levels (Van Winsen *et al.*, 2001). Brooks *et al.* (2001) showed that pigs are tolerant of dietary lactic acid concentrations of up to 200 mmol/kg. In general, acids that are metabolized via the citric cycle (e.g. lactic, citric and fumaric acids) are considered to have a positive effect on feed intake even at relatively high inclusion levels. The effect on palatability always depends on type and dose of the acid. It has been suggested that an improved palatability of a diet can be an important factor for the growth performance of pigs fed organic acids (Brooks, 2008).

The chemical composition of the feed is different depending on where in the fermenting process the feed is, and it affects both pH and palatability (Scholten *et al.*, 1999). Certain fermentation metabolites such as acetic acid and biogenic amides have

been suggested to be the reason for lowering the palatability of a feed, especially in piglets (Brooks *et al.*, 2001; Moran, 2001; Brooks, 2008). Limits have been suggested by several authors. Winsen *et al.* (2001) claimed that the concentration of acetic acid should be below 40 mmol/kg, but Brooks (2003, 2008) and Brooks *et al.* (2003b) claim that a concentration of more than 30 mmol/kg reduced palatability of feeds.

Canibe *et al.* (2010) added acetic acid at 0, 30, 60 and 120 mmol/kg feed and could not see a difference in daily body weight gain or daily feed intake. Biogenic amines are nitrogenous substances that are produced by some LAB strains through a decarboxylation of amino acids. Several authors have seen a higher content of biogenic amines in FLF compared to DF and NFLF which indicates that they are formed during fermentation (Pedersen, 2001). The influence of biogenic amines on the pig's health is unknown, but they cause poisoning in humans at a high consumption. Histamine is one of the most well documented poisonous biogenic amines (Santos, 1996; Spano *et al.*, 2010). The amines reduce feed palatability (Pedersen, 2001; Pedersen *et al.*, 2002) but its effects seem to be poorly investigated. Suarez *et al.* (2010) made a preference test to determine whether pigs preferred some of the acids to the others. They found that the lowest preference was for propionic, acetic, caprylic, formic and butyric acids at the highest inclusion level of their trial, 1.5 % (250 mmol/kg).

2.6.2 Gastrointestinal health

When fermented liquid feed is fed to growing pigs and piglets, it improves the gastrointestinal health of the animals compared to dry feed (DF) or non-fermented liquid feed (NFLF) (Van Winsen *et al.*, 2001b; Canibe and Jensen, 2003; Lindecrona

et al., 2003). The most important health benefits from feeding pigs with an acidified diet or FLF come through the decrease in pH. Pigs fed on FLF have a lower pH in the stomach together with a reduced amount of enterobacteria in the whole gastrointestinal tract compared to pigs fed with dry feed or non-fermented liquid feed (Mikkelsen & Jensen 1998; Van Winsen *et al.*, 2001b; Scholten *et al.*, 2002; Canibe and Jensen, 2003). The pH in the small intestine is usually higher when feeding FLF compared to NFLF. This could depend on the increased production of pancreatic juice as a consequence of the low pH in the gut (Jensen and Mikkelsen, 1998; Canibe & Jensen, 2003; Plumed- Ferrer and von Wright, 2009).

Both fermented feed and acidified feed reduces the incidence of *Salmonella* (Van der Wolf *et al.*, 1999). *Salmonella* control is of high priority in the European pig production and can cause major economic losses through veterinary and hygiene costs as well as lower productivity. It is, therefore, in both the producer's and consumer's interest to prevent the spreading of *Salmonella*. Several serotypes of *Salmonella* are resistant to antibiotics which have made it even more important to prevent contamination. Biosecurity is the most important factor, but a good gut health is effective against *Salmonella*. Van Winsen *et al.*, (2001) showed that the concentration of lactic and acetic acid was responsible for the reduction of *Salmonella* in fermented pig feed. When the concentration of lactic acid was 200 mmol/kg there was a reduction in *Salmonella typhimurium* as acetic acid concentration increased from 10 to 30 mmol/kg. Van Winsen *et al.*, (2001) suggested that it is necessary to have a concentration of 150 mmol/kg lactic acid or 80 mmol/kg acetic acid (with an appropriate pH <4.5) to reduce/eliminate *salmonella* from a feed. Beal *et al.* (2002) found that *Salmonella* died when the level of lactic acid was above 75 mmol/kg and

the pH <4.5. The low pH is crucial, and if a feed is not acidified, either by fermentation or by acidification, *Salmonella* populations multiply in liquid pig feed. It is, therefore, important to acidify the feed even at low temperatures in the control of *Salmonella* (Van Winsen *et al.*, 2001).

2.6.3 Growth performance

Fermented liquid feed has been seen to improve growth performance (Scholten, 1999). Kim (2001) also reported an improvement in DM intake and nutrient utilization when pigs were fed fermented liquid feed. An increase in average daily feed intake is also associated with an increase in average daily gain. Jensen and Mikkelsen (1998) concluded that liquid feeding of pigs resulted in 12 % increase in average daily gain (ADG) in comparison to dry feeding. Improving feed intake during the post-weaning period is very important in enhancing development of the small intestine and subsequent growth performance and in maintaining gut integrity and villus height (Deprez *et al.*, 1987; Pluske *et al.*, 1997), thereby preventing the “growth lag” associated with weaning. The feed to gain ratio was, however, worse for nursery pigs fed LF or FLF compared with dry feed. The poor gain to feed ratio was attributed to increased feed wastage from poor trough design (Russell *et al.*, 1996).

Lawlor and Lynch (1999) reported that even with an improved trough design, pigs fed FLF or LF still had a significantly higher feed conversion ratio compared with those fed dry feed. Originally, one of the major advantages of LF or FLF is that it enabled the use of food industry liquid by-products and biofuel co-products to be incorporate in pig feed. Scholten *et al.* (1999) have demonstrated that pigs fed liquid diet containing fermented by-products showed a significant improvement on average daily gain and

feed conversion ratio compared to pigs on non-fermented liquid diet. Another study involving 1024 pigs, (MLC, 2004) showed that *ad libitum* liquid feeding improved ADG and feed conversion ratio (FCR) by 5.6 and 10.3 %, respectively, with no adverse effect on carcass characteristics.

Most of previous research showing better performance of pigs fed on liquids than dry feeding used wheat and barley based diets. According to de Lange *et al.*, (2006), in a growing-finishing pig performance study, conventional dry feeding was compared to liquid feeding of dry corn or high moisture corn based diets (Table 2.2). Growth performance advantage of liquid feeding was not observed. However, feed efficiency was about 5 % better when pigs were fed high moisture corn. This was in contrast to studies at Stotfold research station from the UK (MLC, 2005). These contrasting findings were related to the liquid feeding equipment (de Lange *et al.*, 2006). At the Stotfold unit, mixed feed was soaked in water in a mixing tank for several hours prior to delivery, while in the Big Dutchman Hydrojet system, feed was dispensed in to troughs within minutes after feed preparation.

The benefit of liquid feeding may be smaller for maize-based diets than wheat and barley-based diets. Soaking may benefit more fibrous ingredients with endogenous phytase activity such as wheat and barley (de Lange *et al.*, 2006). Recent studies indicated that feeding fermented potato pulp had a positive effect on the performance of lactating sows (Xuan Dung *et al.*, 2005). There is, however, little information regarding the use of fermented potato in diets fed to growing-finishing pigs. Hence, more research is required to unveil the advantages that may be available in fermented potato hash based diets.

Table 2.2: Impact of feeding strategy on performance of growing finishing pigs

	Conventional feeding dry pellet feed	Conventional feeding dry pellet feed	Conventional feeding dry pellet feed
Initial BW (kg)	23.5	23.7	23.4
Final BW (kg)	104.7	105.8	104.2
Gain (kg/d)	982	1011	1009
Feed : Gain (88% DM)	2.63	2.64	2.51
Carcass dressing (%)	82.2	80.4	82.5
Carcass lean yield (%)	61.2	60.9	61

Source (Columbus *et al.*, 2006)

2.6.4 Carcass characteristics

There is limited information on how the carcass characteristics of pigs are affected by fermented potato by-products. Urlings *et al.* (1993) demonstrated that feeding fermented feed increased back-fat thickness and decreased meat percentage in finishing pigs. However, replacing 15% of barley in pig diets with pressed beet pulp silage had no effect on carcass parameters (Scipioni and Martelli, 2001). Thomas *et al.* (2013) reported decreased dressing percentage for pigs fed diets containing 40 % potato hash ensiled with or without inoculants. Also, Borton and Rahnema (1998) obtained no differences in carcass traits of pigs fed potato chip scraps. Hang (1998), reports that, the inclusion of 5 % ensiled cassava leaves in the diet of growing pigs did not affect carcass traits.

2.7 Challenges of using liquid feeding

Challenges of using liquid feeding include attaining consistency of by-product, high water content of by-products, huge variability in nutrient content, loss of synthetic amino acids during storage of liquid feeds and achieving homogeneity of mixed feed.

2.7.1 Consistency of by-product supply

It is essential to have formal agreements with by-product suppliers to obtain a consistent quantity and quality of by-products being used. This is important because dry feed premixes and supplements are custom formulated for the specific by-products being used in liquid feeds and because switching between some by-products may reduce growth performance due to the need for the pig's digestive system to adapt to

the changing nutrient composition when switching between by-products (Braun and de Lange, 2004).

2.7.2 High water content of by-products

Many by-products have high moisture (70-90 %). As a result, it is difficult to economically justify transporting liquid by-products long distances due to the high cost per kg dry matter (Braun and de Lange, 2004). Furthermore, the amount of water provided to pigs using liquid feeding is higher than used in conventional dry feeding systems. As a result, manure volume can be increased along with increased humidity and moisture levels in pig facilities.

2.7.3 Variability in nutrient content

Nutrient content of by-products can vary substantially from batch to batch and among sources (Braun and de Lange, 2004). Frequent sampling and nutrient analysis allows for making more precise diet formulation adjustments to avoid feeding excessive or limited amounts of nutrients in liquid feeding systems. Ideally, certificates of quality and nutrient profiles should be obtained from suppliers that guarantee that the by-products are free of contaminants and meet regulatory requirements (Braun and de Lange, 2004).

2.7.4 Loss of synthetic amino acids during storage of liquid feeds

Pedersen *et al.* (2002) showed that about 17 % of added synthetic lysine was lost after 24 hours of storage of fermented liquid feed. This loss is likely due to preferential utilization of free amino acids by microbes found in fermented feeds (de Lange *et al.*,

2006). Niven *et al.* (2006) showed that these losses are primarily due to the presence of coliform bacteria present in fermented liquid feed and that when large amounts of lactobacillus bacteria are present, very little lysine is lost. Therefore, to minimize loss of synthetic amino acids, they should be added to liquid feeds after stable fermentation is achieved, when liquid feed contains more than 75 mMol lactic acid, or when the pH is less than 4.5 (Braun and de Lange, 2004).

2.7.5 Homogeneity of mixed feed

Braun and de Lange (2004) showed that there were substantial differences in mineral content of feed samples collected in some commercial farms they surveyed. They noted that un-mixing of feeds is less of a concern using modern liquid feeding equipment as well as when using higher viscosity liquid by-products such as condensed distillers solubles and corn steep water (by-products of the ethanol industry) to keep mineral particles in suspension longer.

2.8 Key considerations for successful fermentation processes of liquid feed

Fermented liquid feeding creates an opportunity to increase the use of alternative feed ingredients from human food and biofuel industries. Liquid residues from these industries usually contain low dry matter and tend to have variable chemical composition between batches and among samples from different plants. Considering these factors during feed formulation, palatability can be maintained or even increased (Brooks *et al.*, 2001). Unlike the small holder farmer pig production, liquid feeding application in intensive pig production requires suitable equipment in the modern units to maintain the feed in a hygienic and palatable condition (English *et al.*, 1996). Many

reviews of fermented liquid feeding in pigs have been published in the past decade (Jensen and Mikkelsen, 1998; Brooks, 1999; Brooks *et al.*, 2001). Results showed benefits of using fermented liquid feed. Based on growth performance of grower pigs, corn-based diets fed to growing-finishing pigs have shown limited benefit of liquid feeding, in contrast to diets containing wheat and barley (de Lange *et al.*, 2012). These data suggest that fermented liquid feeding is equivalent to feeding pelleted corn-based feeds. When comparing liquid feeding results from different research studies, it is crucial to consider liquid feeding practices such as soaking time, feeding management and feed wastage (de Lange *et al.*, 2012).

The use of microbial fermentation to conserve or improve food is one of the oldest ways of food processing and preservation. Human populations around the world have used microbes to prepare food products for thousands of years and the majority have wide ranges of fermented foods and beverages which contributed significantly to their diets (Achi, 2005).

Brooks (2008) highlighted that increasing feed costs and withdrawal or reduction of antimicrobial growth promoters (AGP) in feeds and quality assurance programmes related to *Salmonella* in pig meat were given as reasons why producers should adopt fermented liquid feeding. The success of fermented liquid feeding depends on: 1. Microbial fermentations and selection of LAB capable of generating lactic acid levels above 100mM that can significantly reduce numbers of *enteropathogens* and the incidence of *Salmonella*. 2. Batch fermentation of the cereal portion of feeds with inoculants capable of generating high lactic acid concentrations to give more consistent results of fermentation. 3. Fermentations that could preserve the feed,

improve the availability of nutrients, reduce the level of anti-nutrients and have LAB with probiotic properties.

Meanwhile, three principal components involved in the fermentation process are the fermenting micro-organisms, the feed substrate and the enabling environment for fermentation (Figure 2.1).

2.8.1 Microbial composition and microbial metabolites of liquid fermented feed

In practice, it is not feasible to ferment a mixture for several days every time fresh feed and water are added before it can be fed to animals. When preparing FLF, fresh feed and water (or another liquid) are mixed with material from the previously successful fermentation, which act as inoculum for the new mixture (back-slopping) (Chae, 2000; Brooks, 2003; Brooks *et al.*, 2003a). That is, fresh feed and water are added to a tank containing a fraction of the fermented mixture to speed up the fermentation and obtain ready-to-feed FLF of good quality a few hours later when it is time for the next feeding.

A temperature in the fermentation tank of ~20 °C, a soaking/fermentation time of 8 h and a residue of 50 % results in FLF (compound feed and water) with pH values of <4.5, low levels of coliform bacteria, high levels of lactic acid bacteria and yeasts, and high levels of lactic acid (Jensen and Mikkelsen, 1998; Canibe and Jensen, 2003). This is considered FLF of good microbial quality. It can be seen that when fermentation starts without any residue in the tank, a period of 3–5 days is needed to reach a steady-state.

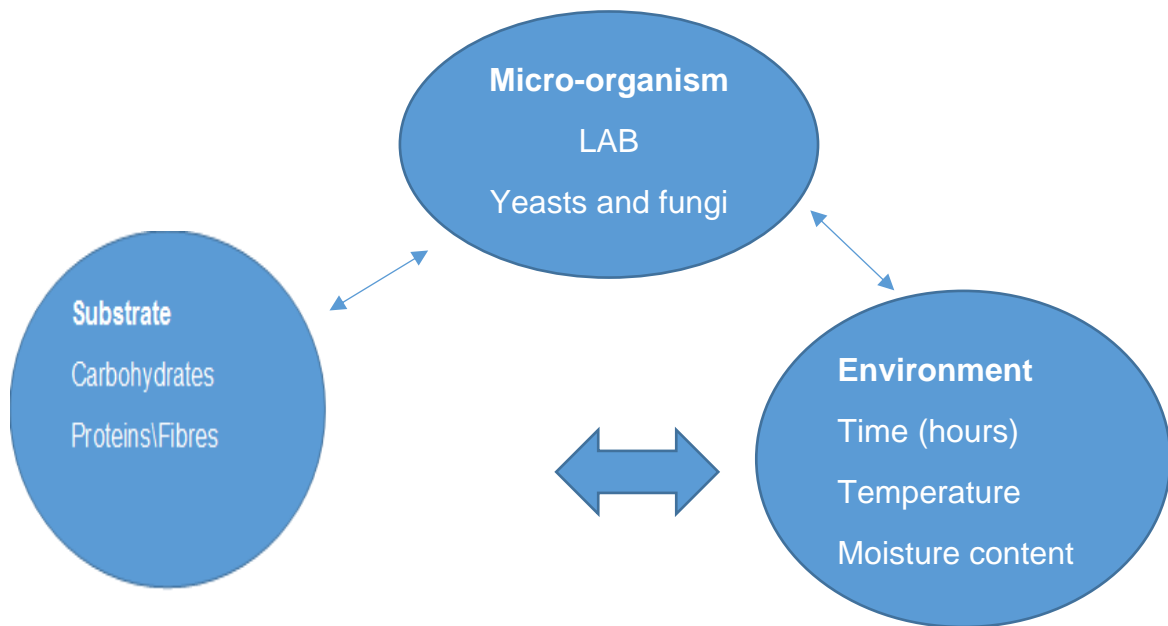


Figure 2.1: Interactions in fermented liquid feed between the micro-organisms present, fermentation parameters and substrate quantity and quality affects the final end product. Adapted from (Niba *et al.*, 2009).

A temperature of 25 °C, residue in the tank of 50 % and 8-h cycles of fermentation are enough to obtain the desired pH (pH lower than 4.5 should be obtained to avoid proliferation of pathogens) (Moran *et al.*, 2006). A temperature of 15 °C is not enough to reach low pH values when the residue is 50 % and the cycles of 8 h of fermentation (de Lange *et al.*, 2012). In principle, and within limits, other combinations of these parameters can be followed to obtain FLF of good quality (for example, higher temperature and smaller residue in the tank) (Canibe and Jensen, 2003).

Moran *et al.* (2006) concluded that there was no apparent advantage in increasing the back-slop proportion, that is, the proportion of previously fermented mixture used as inoculum, above 20 % (33 % and 42 % were tested) in fermented wheat incubated at 24 °C with regard to pH, lactic acid and reduction of coliform counts. The incubation temperature has a big influence on the speed at which a certain pH is reached and on the final pH. Furthermore, Beal *et al.*, (2002) showed big impact temperature has on the biosafety of liquid feed by investigating the survival of *Salmonella typhimurium* DT104:30 in fermented liquid compound feed maintained at 20°C or 30 °C for 72 h. The death rate of *S. typhimurium* DT104:30 was about four times faster in FLF maintained at 30°C compared to FLF at 20 °C. Therefore, the risk period of *Salmonella* spp. contamination of FLF is reduced at higher temperature of incubation.

Some parameters determining the microbial quality of the same feed as dry, non-FLF (feed and water mixed in a bucket, sample taken and analysed within 2 h), or FLF (20 °C, 8 h (day) or 16 h (night), 50 % residual left in the tank, and steady state has been reached) are shown in Table 2.3. Data showed an increase in the number of lactic acid bacteria, but more importantly, a ~30-fold increase of *Enterobacteriaceae* in non-

FLF compared to dry feed (Canibe and Jensen 2003). Further fermentation resulted in inhibition of *Enterobacteriaceae*, and the development of other desirable characteristics (low pH, high lactic acid, and high lactic acid bacteria numbers) as measured in the FLF (Canibe *et al.*, 2007a, Canibe and Jensen 2003). The microbial characteristics of liquid feed during fermentation are very much dependent on time of incubation and incubation temperature. The initial hours are characterized by high pH, low numbers of lactic acid bacteria and yeasts, high numbers of *Enterobacteriaceae*, and low concentration of lactic acid (Brooks et al., 2003a). Whereas after further hours of incubation, the pH decreases, the number of lactic acid bacteria and yeasts, and the concentration of organic acids and ethanol increase, whereas the *Enterobacteriaceae* counts decrease (Canibe and Jensen, 2003).

Table 2.3: The pH, microbial and chemical composition of dry feed (DF), non-fermented liquid feed (non-FLF) and fermented liquid feed (FLF)^a

	DF	Non-FLF ^b	FLF ^c
pH	NM ^g	5.98±0.18	4.36±0.17
Lactic acid bacteria (log cfu/g)	<4.3±0.86	7.2±0.18	9.4±0.32
<i>Enterobacteriaceae</i> (log cfu/g)	<4.7±1.10	6.2±0.59	<3.2±0.56
Yeasts (log cfu/g)	<3.6±0.59	5.0±0.65	6.9±0.69
Lactic acid (mM)	0	1.2±2.31	169±17.07
Acetic acid (mM)	28±1.98	2.3±2.37	25.8±6.32
DM (g/kg)	893±3.8	273±12.0	246±8.8
Protein (N×6.25) (g/kg DM)	177±13.3	171±09.8	176±1.4
Lysine (g/16 g N)	6.0±1.3	5.8±1.14	4.8±0.87
LMW-sugars ^d (g/kg DM)	36±1.3	29±5.8	1±0.6
Starch (g/kg DM)	460±9.0	468±16.8	465±24.2
I-NSP ^e (g/kg DM)	110	111±7.8	96±4.5
T-NSP ^f (g/kg DM)	127	133±7.3	117±0.0

^aAdapted from Canibe and Jensen (2003). ^bNon-heated compound feed and water (1:2.5, w/w) mixed in a bucket, sample taken and analysed within 2 h, ^cNon-heated compound feed and water (1:2.5, w/w) incubated at 20 °C, soaking time of 8/16 h and a residue of 50%. ^dLow molecular weight sugars. ^eInsoluble non-starch polysaccharides. ^fTotal non-starch polysaccharides. ^gNot measured.

Since microbial growth is affected by temperature, substrate and contamination from the environment, the microbial composition of liquid feed can be expected to be affected by factors like time of fermentation, fermentation temperature, location where the fermentation is carried out, and composition of the feed/ingredient to be fermented (Canibe and Jensen, 2003). Various authors have investigated some of these factors in relation to microbial composition of FLF to species level. Canibe *et al.*, (2007a) analysed terminal-restriction fragment length polymorphism (T-RFLP) profiles in fermented liquid cereal grains, liquid compound feed containing fermented liquid cereal grains, and FLF prepared with a standard pig diet. The presence in all samples measured of a fragment tentatively identified as *Lactobacillus plantarum* confirmed previous results (R. Engberg and B.B. Jensen, unpublished data). Using 16S rRNA gene sequencing, these authors reported *L. plantarum* to be the most frequently isolated species in FLF. Shlimon *et al.*, (2006), however, clearly indicated *Pediococcus pentosaceus* as the most dominant bacterium in FLF prepared with standard pig compound feed and in fermented cereal grains (50 % barley and 50 % wheat). In addition, Shlimon *et al.*, (2006) showed that the composition of the lactic acid bacteria population changed with time of fermentation. After 48 hours of fermentation, *Weissella* species dominated, whereas at 180 h of incubation, and after backslopping had been practised, *Ped. pentosaceus* made up 74–88 % of the total isolates identified in both samples.

Changes in microbial population with time of fermentation were also observed by Olstorpe *et al.* (2008) (Table 2.4). These authors mixed cereal grains with whey, with water or with wet wheat distillers' grains and practised backslopping daily from day five. In the sample mixed with whey, *L. plantarum* dominated during the 19 days of

fermentation the study lasted. In the two other samples, *Ped. pentosaceus* dominated during the initial days, while *L. plantarum* started to proliferate during the last days of fermentation and after backslopping had been practised. In sample containing cereals and whey, a shift with time of incubation in the yeast population from a dominance of *Kluyveromyces marxianus* to *Pichia membranifaciens* was measured. In the mixture of cereals and water, *Pichia anomala* was initially the dominant species, but after feed replacement, *Saccharomyces bayanus* and *P. membranifaciens* were also detected. Microbial population dynamics with time of fermentation can be explained by a better acid tolerance and a metabolism adapted to the environment. For example, by possessing the capability for fine-tuning reactions to equilibrate the redox balance, of some microorganisms (van der Meulen *et al.*, 2007). These fine-tuning reactions might give an extra competitive advantage to the strains possessing this capability (van der Meulen *et al.*, 2007).

2.8.2 Influence of micro-organisms and feed substrates on fermented liquid feed

The selection of LAB for feed fermentation to meet desired feed and production objectives has been highlighted in previous reviews (Brooks *et al.*, 2003b). The choice of feed substrates to obtain high numbers of LAB (log 9 cfu/g feed) and levels organic acids (> 150 mM) or a consistent fermentation product has also been researched (Canibe *et al.*, 2007a; Lyberg *et al.*, 2008; Olstorpe *et al.*, 2008). Fermentation objectives that have influenced the use of LAB have centred on: 1. Selection for rapid production of organic acids (mainly lactic acid) to ensure biosafety (e.g. Missotten *et al.*, 2007; Missotten *et al.*, 2008). 2. Selection for homolactic fermentation to improve feed palatability. 3. Breakdown of anti-nutrients and increased bioavailability of

nutrients (Bertsch *et al.* 2003; Brooks *et al.* 2003a; Oboh 2006; Skrede *et al.*, 2007; Lyberg *et al.*, 2008; Okpako *et al.*, 2008) (Table 2.4).

2.9 Strategies to improve the microbial and nutritional characteristics of fermented liquid feed

Liquid feed needs to be adequately fermented in order to obtain a product of optimal and stable microbial quality. Nutritional characteristics can be impaired due to this fermentation. Moreover, pigs fed FLF have shown lower feed intake compared to those fed non-FLF (Pedersen, 2001; Canibe and Jensen, 2003). The lower feed intake on FLF has been speculated to be due to high concentration of acetic acid and biogenic amines, by impairing palatability (Brooks *et al.*, 2001). Several strategies can be used to avoid the detrimental characteristics fermentation can give to the mixture being fermented.

Table 2.4: Effects of micro-organisms and feed substrates on fermentation

Fermentation type	Substrate	pH	LA	AA	Ethanol	Source
<i>L. plantarum</i> , <i>P. pentosaceus</i> , Yeasts	Wheat and wheat by-products	<3.8 4.53	<60 [†] 22.21*	<10 [†] 22.42*	<10 [†] 10.74*	(Moran <i>et al.</i> , 2006) (Beal <i>et al.</i> , 2005)
Spontaneous fermentation. (LAB and yeasts)	Barley	4.30	34.43*	27.34*	10.74*	(Beal <i>et al.</i> , 2005)
LAB fermentation	Phytic acid in cereals	-	Increase in apparent bioavailability of Phosphorus, Calcium, Magnesium and Copper			(Brooks <i>et al.</i> , 2001, Brooks 2008)
<i>Aspergillus niger</i> and <i>L. rhamnosus</i>	Cassava peel meal	-	Increase in protein (24.4%), ash (7.5%), crude fibre (10.62%) and decrease in cyanide (7.35 mg/kg)			(Okpako <i>et al.</i> , 2008)
<i>Saccharomyces cerevisiae</i> and <i>Lactobacillus</i> spp.	Cassava peel meal	-	Increase in protein content (21.5%) and decrease in cyanide (6.2 mg/kg), and phytate (789.7 mg/100g).			(Obboh 2006)

[†] g kg dry matter. * mmol/L. LA- Lactic acid, AA- Acetic acid

2.9.1 Addition of starter cultures

The purpose of using starter cultures during preparation of FLF is to improve: (1) its nutritional quality and palatability by decreasing/avoiding the degradation of lysine and reducing the level of acetic acid and biogenic amines; and (2) its microbial quality by accelerating the production of, mainly, lactic acid and consequently, reducing the pH and avoiding the proliferation of coliforms and other possible pathogens in the mixture (Brooks *et al.*, 2001). Cultures can also be used to degrade or bind anti-nutritional or toxic compounds present in feed ingredients or produced during fermentation (Shetty and Jespersen, 2006; Niderkorn *et al.*, 2007; Holzapfel, 2010), but this aspect has only scarcely been explored in the field of feeding FLF to pigs. Brooks *et al.* (2001) observed a reduction in the level of acetic acid and almost no presence of cadaverine in compound liquid feed during the initial 48 h of incubation by adding a lactic acid bacteria strain. Whether this effect was also seen in later stages of fermentation, that is, once the natural microflora had been established, was not shown in that study. Furthermore, Niven *et al.* (2006) showed that addition of *L. plantarum* and *Ped. acidilactici* resulted in higher concentration of lactic acid, impeded a blooming of coliform bacteria, and impeded lysine degradation and cadaverine production in liquid feed during the initial 72 h of incubation at 35 °C compared to an uninoculated sample. Again, whether the same effects would be observed in later stages of fermentation was not investigated. Also Missotten *et al.* (2007, 2009) found out that several lactic acid bacteria were able to accelerate the pH drop and contribute to a higher lactic acid concentration during 72 h of incubation compared to uninoculated liquid feed. Several reports have shown a more rapid pH drop, higher counts of lactic acid bacteria, and a reduction of the blooming of coliforms during the initial hours of incubation after addition of a starter culture to the mixture compared to uninoculated FLF (Jensen and

Mikkelsen, 1998; Canibe *et al.*, 2001; Plumed-Ferrer *et al.*, 2005). Once the spontaneous microflora presence in the feed and/or environment had been established, a small or no effect of starter culture addition on the same parameters could be observed.

2.9.2 Addition of acids

Addition of organic acids to liquid feed in order to improve its biosafety by impeding the proliferation of *Enterobacteriaceae* is a relatively common practise (Niven *et al.*, 2006). The antibacterial activity of organic acids is related to the reduction of pH in the liquid feed, and their ability to dissociate, which is determined by the pKa-value of the respective acid, and the pH of the surrounding milieu (Cherrington *et al.*, 1991). The undissociated acid can enter the bacteria, where it releases the proton in the more alkaline environment, resulting in a decrease of intracellular pH (Shetty and Jespersen, 2006; Niderkorn *et al.*, 2007; Holzapfel, 2010). This influences microbial metabolism inhibiting the action of important microbial enzymes and forcing the bacterial cell to use energy to release protons, leading to an intracellular accumulation of acid anions (Cherrington *et al.*, 1991).

A frequently used acid is formic acid. The acid or its salt is added directly to the mixture as it is or via other products containing formic acid such as whey. Canibe *et al.* (2001) added formic acid to liquid compound feed to reach concentrations of 1, 3, 5 and 10 g/kg of liquid feed. The liquid feed added 1 g/kg formic acid contained somewhat lower counts of lactic acid bacteria during the initial 48 h of incubation, and importantly, no blooming of *Enterobacteriaceae* was observed. Addition of 3 g/kg or more formic acid also impeded the blooming of *Enterobacteriaceae* but it implied that fermentation was

inhibited, that is, lactic acid bacteria did not proliferate and organic acids were not produced. The addition of formic acid at a concentration of 1 g/kg impedes the proliferation of the undesirable bacteria (*Enterobacteriaceae*) allowing the desirable bacteria (lactic acid bacteria) to grow, whereas levels of 3 g/kg or higher do not allow fermentation to occur. In the study of Canibe *et al.* (2007b), it was shown that addition of 2 g/kg formic acid to a liquid compound pig feed impeded a blooming of *Enterobacteriaceae* but the proliferation of lactic acid bacteria was also considerably reduced. In an attempt to limit lysine degradation in liquid compound feed during fermentation, by preventing the growth of *E. coli*, Niven *et al.* (2006) added 50 mM lactic acid to the mixture and after 72 h of incubation at 35 °C, there was no evidence of lysine metabolism.

van Winsen *et al.* (2001a), however, showed how adding lactic acid and acetic acid to FLF decreased the survival of *S. typhimurium*. Canibe *et al.* (2007b) measured the impact of adding 2 g/kg formic acid or 4.8 g/kg Boliflor® FA2300 S (a solid feed acidifier containing 251 g formic acid/kg, 151 g ammonium formate/kg, 25 g potassium sorbate/kg) to liquid compound feed. Although addition of the products reduced, but did not eliminate, the number of *Enterobacteriaceae* compared to the control treatment, no difference in degradation of free lysine after backslopping had been practised was seen. Hence, the reduction of *Enterobacteriaceae* in FLF to the extent reached in that study was not effective avoiding or attenuating the degradation of lysine. In summary, the addition of organic acids to liquid feed can be used as a means of increasing the biosafety and nutritional quality of FLF. However, too high doses of acids can impede fermentation of the mixture to take place. A balance between desirable effects of

organic acid addition and those of fermentation itself should be achieved. The economical aspect related to the addition of acids should also be taken into account.

2.9.3 Addition of enzymes

Since enzymes need water to be active (Carlson and Poulsen, 2003), addition of enzymes to liquid feed gives the possibility of enzyme activation outside the gastrointestinal tract of the animals before consumption. This strategy can be a means of further improving the digestibility or fermentability of nutrients. Literature on the impact of exogenous enzyme addition to liquid feed on digestibility of nutrients is limited. Carlson and Poulsen (2003) showed how addition of phytase to compound feed increased the degradation of phytate with time of fermentation. The biggest effect was clearly seen when cereals were heat-treated (which inactivated the endogenous phytase). Temperature had a big impact on the speed of the reaction, i.e., at higher temperature, there was faster degradation of phytate. Blaabjerg *et al.* (2007) observed no effect of phytase addition to wheat on phytate degradation during 8 h of incubation, whereas addition to soybean meal increased phytate degradation from ~18 % to ~90 % after 8 h of incubation. Similar data were reported by Brooks *et al.* (2001), Christensen *et al.* (2007) added xylanase and -glucanase to liquid feed. After 8 h of fermentation, the material was subjected to *in vitro* digestion, and increased solubilization of fibre (NSP) and DM were measured in the enzyme supplemented liquid feed compared to the non-supplemented feed. Fermentation during 8 h itself increased NSP and DM solubilization, too, but the highest effect was observed when a high enzyme dose was added to liquid feed. These effects may be of importance since solubilization of NSP is a prerequisite for an efficient hindgut fermentation of these components in pigs. On the contrary, Choct *et al.* (2004) registered a negative

impact of xylanase addition to liquid feed fermented during 1 h on energy digestibility in weaner pigs and no effect when the feed was fermented during 15 h.

Addition of ingredients containing high levels of phytase, e.g., brewer's yeasts, has also been reported to increase phytate degradation of a maize/soybean meal based-diet during 24 h soaking (Chu *et al.*, 2009). Brooks *et al.* (2001) presented data on in vitro digestibility of soyabean meal steeped for 24 h with three different proteases. All three enzymes improved the digestibility from 0.758 without enzyme addition to 0.858–0.889 % in the samples added protease. In summary, addition of enzymes to liquid feed has the potential to significantly increase the degradation of fibre during soaking/fermentation. However, the ingredient or mixture of ingredients, as well as, their processing before fermentation has a big impact on the results obtained. On the other hand, the influence of addition of other enzymes to feed on its nutritional quality has generally shown positive results, but it has, so far, only been scarcely investigated. Besides the impact of enzyme addition during soaking, more data on the influence of adding enzymes to an established FLF system are needed.

2.10 Controlled fermentation using cultures

Successful fermentation results have also been found to be dependent on the type of fermentation adopted. Spontaneous (Beal *et al.*, 2005), backslopping (Moran *et al.*, 2006), inoculated or controlled fermentations (Christensen *et al.*, 2007; Canibe *et al.*, 2008) have been investigated as methods that could be used for production of fermented feeds.

Spontaneous fermentation has been discouraged (Brooks *et al.*, 2003b; Brooks, 2008) because in this system yeast, which can tolerate low pH and a low temperature, can predominate. Yeast fermentation of starch will result in alcohol and carbon dioxide production. The production of CO₂ represents a loss of feed dry matter and energy value. Such feeds could be unpalatable due to 'off' flavours resulting in reduced feed intake. Secondly, spontaneous fermentation may not guarantee a rapid build-up of lactic acid in the feed, which is necessary for biosafety of the feed and to limit the pathogens (Niven *et al.*, 2006). Lastly, since feed ingredients differ in their load of natural microflora, spontaneous fermentation of the same raw material at different times results in inconsistent end products.

Backslopping has been practiced on many farms (Beal *et al.*, 2002). The limitations of this method have recently been highlighted in the review by Brooks (2008). In addition to these limitations, additions of fresh feed to a dynamic fermenting medium could have adverse implications on microbial balance and the ability of the feed to resist enteropathogens. Temperature shifts during fermentation that are outside the optimal range of particular pathogens could provoke the secretion of cold-shock proteins (Beal *et al.*, 2002). Such cold shock proteins could increase pathogen tolerance to lactic acid in feed fermented at 20°C compared with 30°C. Controlled fermentation or inoculated fermented liquid feed would appear preferable for production of fermented liquid feeds for pigs or fermented moist feeds because more predictable results could be obtained (Beal *et al.*, 2002). Selection for LAB that produce lactic acid rapidly, with high 24 h lactic acid (>150 mmol/L) contents (Brooks, 2008), should be the primary objective.

2.11 Desired properties of fermented liquid feed

When feed is soaked in water for a certain length of time, lactic acid bacteria and yeasts naturally occurring in the ingredients proliferate and produce mainly lactic acid and acetic acid, which reduces the pH of the mixture ultimately preventing the proliferation of spoilage organisms and foodborne pathogens (Nout *et al.*, 1989; Russell and Diez-Gonzales, 1998; van Winsen *et al.*, 2001a). The *Enterobacteriaceae* are usually active when incubated at 20°C and reach maximum levels at 24 h, but with decreasing pH their level decreases as a result of increasing organic acids (Canibe and Jensen, 2011). Fermented Liquid Feed is assumed to be best when the fermentation, spontaneous or induced, yields stable and high numbers of LAB, stable and low pH (3.5–4.5), and consequently low or non-existent *Enterobacteriaceae* population (Geary *et al.*, 1999; Brooks *et al.*, 2003; Plumed-Ferrer *et al.*, 2005).

According to Canibe and Jensen (2003), the initial phase of fermentation is characterized by low levels of LAB, yeasts, and lactic acid, high pH, and, importantly, a blooming of enterobacteria. This phase is followed by a second phase, in which a steady state is reached, and also characterized by high levels of lactic acid bacteria, yeasts, and lactic acid, low pH, and low enterobacteria counts. Van Winsen *et al.* (2000) also showed that lactic acid is the main organic acid responsible for the antimicrobial effect of FLF. A fermentation product dominated with yeast is not desirable because it reduces feed intake (Plumed-Ferrer *et al.*, 2008). Due to the production of high mixtures such as acetic acid, ethanol and amylic alcohols, FLF might have an unpleasant odor and/or taste (Brooks *et al.*, 2003a; Canibe *et al.*, 2007). Jensen and Mikkelsen (1998) found an inverse relationship between the concentration of yeast and enterobacteria in the gastro intestinal tract (GIT) of pigs. Therefore, high

concentrations of yeasts in the FLF may also be beneficial. However, the population diversity of yeasts present in FLF is very high and deserves further investigation (Olstorpe *et al.*, 2008).

2.11.1 Influence of fermentation length

The length of steeping feed ingredients, the type of feed substrates and fermentation conditions influence the quality of the fermentation product (Brooks *et al.*, 2003a). Steeping time has been related to its effects on the activity of endogenous enzymes and the breakdown of anti-nutrients within the feed (Brooks *et al.*, 2003a). According to Choct *et al.* (2004a) the effects on growth and feed intake for weaner pigs resulting from steeping of feed for 15 hours might be related to the release and activation of endogenous enzymes in the feed. The activation of these enzyme systems within the feed can act on cell wall structures in a similar way to exogenous feed enzymes (Choct *et al.*, 2004b).

In reviewing the effect of steeping in liquid feeding systems, Brooks *et al.* (1996), indicated that phytases that were naturally present in the pericarp of some grains (like cereals) could be activated by soaking. They also stated that soaking feed for 8-16 h before feeding increased the bioavailability of phosphorus, calcium, magnesium and copper. In another study (Lybcrg *et al.*, 2008), the phytase activity for a cereal grain mix of wheat, barley and triticale was 1382 FTU /kg DM and inositol hexaphosphate bound-phosphorus and total phosphorus were 2.2 and 3.7 g/kg DM. After fermentation, dietary inositol hexaphosphate was completely degraded to release phosphorus. The fermentation of carbohydrate-rich cereal components of the diet

separately and combining them with the protein-rich components just before feeding is necessary (Bcal *et al.*, 2002; Moran *et al.*, 2006; Canibe *et al.*, 2007a; Brooks, 2008).

The main goal of fermentation is a high lactic acid concentration ($> 150\text{mmol/L}$) and a low pH (<4.5) (Brooks 2008). Temperature affects fermentation rate and low temperatures may yield insufficient quantities of fermentation end-products (Brooks 2008). Fermentation of a cereal grain mix at 10°C produced 8.6 g^{-1} of lactic acid compared with 13.6 g^{-1} at 20°C (Lyberg *et al.*, 2008). At low temperatures, yeast predominates and produces ethanol (Brooks, 2008). Insufficient lactic acid concentration with 24-hour fermentation cycles which are more practical on farms may be the case at low temperatures (Brooks 2008). Prolonged fermentation also results in considerable variation in species composition of fermented pig feed (Olstorpe *et al.*, 2008). However, fermentation at 30°C seems ideal as at 35 and 40°C there was no significant effect on lactic acid concentrations and acetic and butyric acid and ethanol concentrations were significantly increased (Beal *et al.*, 2005). Some of the effects of incubation time and temperature on fermented feeds properties are presented in Table 2.5.

Table2.5: The effects of fermentation length and conditions

Incubation (hrs)	Temp (°C)	pH	LA effects on diet	AA effects on diet	Ethanol effects on diet	Source
24	-	3.75 [†]	54.5	Yeast population increased 10 fold		(Moram <i>et al.</i> , 2006)
48	-	3.65	Yeast population stabilizes and coliforms eliminated mainly by back-slopping			
24	-	4.69	11.68*	17.22*	6.81*	(Beal <i>et al.</i> , 2005)
48	-	4.34	31.92*	27.55*	10.62*	
72	-	4.21	46.14*	30.75*	12.79*	
-	30	4.41	30.16*	16.42*	11.69*	
-	35	4.36	25.29*	26.57*	9.78*	(Beal <i>et al.</i> , 2005)
-	40	4.2	28.64*	32.89*	8.42*	
48	20	3.9	115*	D _{value(min)} -250		
72	20	3.8	164*	D _{value(min)} -164		
96	20	3.8	167*	D _{value(min)} -137		
48	30	3.8	161*	D _{value(min)} -45		
72	30	3.8	196*	D _{value(min)} -38		
96	30	3.8	203*	D _{value(min)} -34		
1	Room	-	Increase in phosphorus	apparent digestibility	of	(Lyberg <i>et al.</i> , 2005)
0 ^a	10		ND ^b	5.5 ^c		(Olstorpe <i>et al.</i> , 2008)
0 ^a	20		ND ^b	5.5 ^c		
3 ^a	10		ND ^b	ND ^c		
3 ^a	20		ND ^b	5.6 ^c		
5 ^a	10		ND ^b	3.8 ^c		(Lyberg <i>et al.</i> , 2008)
5 ^a	20		3.1	4.9 ^c		
7 ^a	10		2.0 ^b	4.5 ^c		
7 ^a	20		2.1 ^b	6.2 ^c		
17-19 ^a	10		1.8	10.4	1.2	
17-19 ^a	15		1.9	10.4	1.2	
17-19 ^a	20		2.2	10.5	1.1	

[†]g/kg dry matter, *mmol/L, Dvalue (min)-decimal reduction time (minutes) of Salmonella in fermented feed, ^adays, ^byeasts counts (cfu/g feed), ^cLAB counts (cfu/g feed), ^dEnterobacteriaceae counts (cfu/g feed), dmoulds, ND-not detected.

2.11.2 Water to feed ratio

An important feature of a successful liquid feeding system is the water to feed ratio of the pig diet. This affects the DM content of the diet and may also influence the intake and organic acid concentration of the feed. Research to confirm the ideal DM content of liquid diets is limited (Choct *et al.*, 2004a). Generally, the water to feed ratio of LF or FLF can fluctuate between 1.5:1 and 4:1 (Brooks, 2008). Barber *et al.* (1991) as shown in Table 2.6 reported that DM digestibility increased linearly with increasing water to feed ratio from 2:1 to 4:1.

Gill *et al.* (1987) reported that decreasing water to feed ratio also had a beneficial effect on growth rate and feed conversion ratio. It has been suggested that the change of water to feed ratio results in a reduction of viscosity of the digesta, and hence, allows for more contact between digesta and digestive enzymes (Brooks, 1999). Geary *et al.* (1996) also studied the performance of weaner pigs fed diet mixed with water to provide four different DM concentrations of 149, 179, 224 and 255 g kg⁻¹, over a four-week period post-weaning. They concluded that weaner pigs would readily accept liquid feed with DM content in the range 255 to 149 g kg⁻¹ and piglets on liquid diets were able to control dry matter intake through increasing total volumetric intake and regulating the voluntary water intake from nipple drinkers.

Table 2.6: Effect of water to feed ratio on diet digestibility

	Water to feed ratio			
	2 :1	2.67: 1	3.33:1	4 :1
Dry matter digestibility (%)	79.1 ^a	77.8 ^a	80.3 ^{a,b}	82.9 ^b
Estimated DE (MJ Kg-1) DM	15.1	14.9	15.4	15.8

^{a,b}Values within a row without common superscripts differ (p < 0.05).

(Barber *et al.*, 1991)

2.12 Summary

Fermented liquid feeding has a potential advantage for feeding grower pigs. Fermented liquid feeding has been reported to alleviate the nutritional stress due to lack of feed ingredients and availability of by-products. Most of the research on liquid feeding has been based on wheat and barley diets. With the adoption of automatic pig

liquid feeding systems there had been tremendous value in pig production, especially after the ban of antibiotics use. The liquid system is helpful in improving management and health status of pigs. In addition, liquid feeding has been using cheap by-products from food industries, candy manufacturing, milk processing (whey, butter milk) and beakers and beverage industries. With the increasing production of by-products (liquid / wet), there is greater potential to introduce and exploit the benefit of using fermented liquid feed which ultimately may reduce cost of feed and increase production and profit.

2.13 References

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Chapter 3

Nutritive value and physical properties of varying levels of fermented liquid potato hash diet treated with or without exogenous enzyme

Abstract

The present study aimed to characterise physicochemical properties and nutritive value of different inclusion levels of fermented liquid potato hash with or without added exogenous enzyme. Different inclusion levels of potato hash were mixed with water at a ratio of 1:2 to ferment for 8 hr. An exogenous xylanase enzyme (Natugrain TS L®) was added at 5 % to the feed before fermenting the diets. A back-slop fermentation process was followed. Diets were formulated to provide 14 MJ/kg digestible energy (DE), 180 g crude protein (CP)/kg and 11.6 g lysine/kg. Diets were: CON (control diet that is not fermented and without potato hash), LFC (fermented control diet), LLPH (diet containing 200 g potato hash.kg⁻¹ diet, as fed), HLFPH (diet containing 400 g potato hash.kg⁻¹ diet, as fed), LFCE (fermented control diet treated with an exogenous xylanase enzyme (Natugrain TS L®), LLFPHE (diet containing 200 g potato hash.kg⁻¹ diet treated with the exogenous xylanase enzyme, HLFPE (diet containing 400 g potato hash.kg⁻¹ diet treated with an exogenous xylanase enzyme. The LLFPH+E and HLFPH+E diets had a higher ($P < 0.05$) DM, CP, WHC and lower ($P < 0.05$) NDF, ADF and SC compared to LLFPH and HLFPH diets. However, there was no difference ($P < 0.05$) on EE, GE and density among the diets. The addition of the fibrolytic enzyme at both low and high liquid fermented potato hash reduced fibre content of the feed. Further work is needed to investigate the effects of liquid fermented potato hash diets on digestibility of nutrients and feed intake.

Keywords: dietary fibre; potato hash; Fermented liquid feed; enzyme.

3.1. Introduction

Potato hash (PH), a by-product derived from the processing of snacks and chips, is produced at \pm 50 tons per day in South Africa and is currently dumped or given to farmers (Nkosi and Meeske, 2010). Utilizing PH to replace imported commercial feedstuffs could possibly reduce environmental impact of burning it to wastes or dumping as landfill (Nkosi, 2009). The by-product contains a dry matter (DM) of 150 g/kg, 700 g/kg DM of starch, 105 g/kg DM of Crude Protein, and crude fibre of 58.5 g/kg DM (Nkosi, 2009) and a relatively small amount of yellow maize and fats. One of the limitations for using potato by-products in pig nutrition is their high moisture content, which makes transportation costly (Okine *et al.*, 2005; Charmley *et al.*, 2006).

Another setback in feeding potato hash to pigs is that it contains low DM and water-soluble carbohydrates (WSC) contents (Okine, 2007) and high fibre. High fibre in pig feed poses a challenge in that it increases rate of feed passage in the pig gut and sequesters nutrients in the fibre matrix reducing their digestion (Stanogias and Pearce, 1985; Fevrier *et al.*, 1992). It has, however, been shown that fermentation and use of exogenous enzymes, strong acids and bases disrupt the fibre matrix structure enabling further breakdown of fibre components (Latif and Rajoka, 2001; Le Gall *et al.*, 2009; Zhang *et al.*, 2010; Urriola *et al.*, 2010). Safety, environmental and economic concerns preclude the regular use of strong acids and bases to improve potato by-products as pig feed.

Additives have been successfully added to various high moisture by-products to reduce DM losses and improve nutritive value (Urriola *et al.*, 2010). Enzymes have been widely used as additives (Nkosi *et al.*, 2015, 2009). Cell wall degrading enzymes

improve chemical characteristics and reduce fibre content in fermented sorghum straw (Meeske *et al.*, 1999; Colombatto *et al.*, 2004; Xing *et al.*, 2009). In principle, exogenous enzymes degrade fibre to fermentable WSC for use by LAB since these organisms cannot use fibre as an energy source (Eun and Beauchemin, 2007). The presence of LAB may, however, inhibit exogenous enzymes' activities against structural carbohydrates in fermented feed (Stokes, 1992; Xing *et al.*, 2009). Commercial xylanases could degrade xylans under anaerobic conditions, leading to reduction in other fibre components thus improving the nutritive value.

Physicochemical characteristics of fibrous feeds play a key role in feed intake of grower pigs. The physicochemical properties of fibrous feeds include water holding capacity (WHC), bulk density (BD), acid detergent fibre (ADF), neutral detergent fibre (NDF) and crude fibre (CF). The physicochemical characteristics vary across fibre sources (Ndou, 2012). Fibres with a high WHC, for example, will absorb more water, swell and occupy more space in the stomach. Consecutive stimulation of stretch receptors due to distension of the gut will lead to satiety (De Leeuw, 2004). Satiety is the state of being satisfactorily full and unable to take on more feed. This affects feed intake and, consequently, performance of growing pigs.

Whittemore *et al.* (2002) conducted a study with few fibrous sources with a narrow range of physicochemical properties. Use of more fibrous feedstuff at varying inclusion levels provides a wider variation of physicochemical properties to generate more accurate predictions of feed intake in growing pigs. The objective of the current study was to characterise physicochemical properties and nutrient composition of varying levels of fermented liquid potato hash diets treated with or without an exogenous

xylanase enzyme. It was hypothesized that the physicochemical properties of varying levels of fermented liquid potato hash diet are highly variable.

3.2. Materials and Methods

Potato hash (PH) was collected from Simba (336 Andre Greyvenstein road, Isando, Gauteng, South Africa), a local food producing factory in South Africa for fermentation and proximate analysis. Exogenous xylanase feed enzyme (Natugrain TS L®) was obtained from Danisco Ltd (Tsessebe Crescent, Midrand, South Africa). Natugrain TS, the feed enzyme product from BASF, contains the highly purified NSP-splitting enzymes endo-1,4-beta-xylanase and endo- 1,4-beta-glucanase activity of 5600 TXU¹./g and 2500 TGU²./g (¹*Thermo-stable Xylanase Unit*; and ²*Thermo-stable Glucanase Unit*).

3.2.1 Preparation of fermented potato hash treated with or without enzyme and sampling

3.2.2.1 Treatments

Seven diets were formulated to be isoenergetic and isonitrogenous containing different levels of Liquid fermented potato hash (LFPH): 0, 200, 400 g kg⁻¹ DM treated with or without enzyme. These diets were formulated to provide 14 MJ/kg digestible energy (DE), 180 g crude protein (CP)/kg and 11.6 g lysine /kg which meet and exceed the requirements of growing pigs (NRC, 1998). The seven dietary treatments were: CON (control diet that was not fermented and without potato hash), LFC (liquid fermented control diet), LLPH (diet containing 200 g potato hash.kg⁻¹ diet, as fed), HLFPH (diet containing 400 g potato hash.kg⁻¹ diet, as fed), LFCE (fermented control diet treated with an exogenous xylanase enzyme (Natugrain TS L®), LLPPHE (diet containing 200

g potato hash.kg⁻¹ diet treated with an exogenous xylanase enzyme (Natugrain TS L®), as fed), HLFPE (diet containing 400 g potato hash.kg⁻¹ diet treated with an exogenous xylanase enzyme (Natugrain TS L®).

3.2.2.2 Fermentation of diets and experimental design

The back-slopping fermentation approach was followed as described by Plumed-Ferrer and Von Wright, (2009). Fermented liquid diets were prepared by mixing diets with water, at a ratio of 1:2 for 8 hr. An exogenous xylanase enzyme (Natugrain TS L®), as fed was added at (0.5 g/kg-1) before fermentation of diets (Table 3.1). The fermented diets were stored in a closed 100 L drum under agitation at 25°C. After 8 h, 50% of the content was taken out and replaced in the drum with an equal amount of fresh feed and water. Feed samples were taken in triplicates for determination of dry matter, gross energy (GE), crude protein (CP), ether extract (EE), water holding capacity, bulk density, swelling capacity, neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin analyses.

3.2.2.3 Chemical composition

Liquid fermented potato hash with or without enzymes were analysed, in triplicate, in the Animal and Poultry Science Laboratory at the University of KwaZulu-Natal, Pietermaritzburg. Dry matter (DM) content was determined by oven-drying the samples at 65 °C for 48 hours. The ash content was determined after incineration of the sample at 550 °C for 4 h according to method 990.05 (AOAC, 1990). Dry samples were ground through a 1 mm screen (Wiley mill, Standard Model 3, Arthur H. Thomas Co., Philadelphia, PA, USA) for chemical analyses. The DM was determined by drying samples at 60 °C until a constant mass was achieved and corrected for loss of

Table 3.1: Composition of the diet on as-is basis of different inclusion levels of liquid fermented potato hash

Ingredient, kg	Experimental diets ¹		
	CON	LLFPH	HLFPH
Hominy Chop	608.7	504.4	400
Molasses	20	15	10
Potato hash	0	200	400
Soybean oilcake	181.4	166.7	152.1
Corn meal	150	75	0
Monocalcium phosphate	5	8.1	11.2
Limestone	18.8	16.3	13.7
Lysine HCl	8	6.5	5
Salt	4	4	4
Vitamin–mineral premix ²	4	4	4
Calculated composition			
Nutrients, g/kg			
DM	89.2	60.5	59.9
Ash	2.5	3.1	3.7
CP	16.8	16.3	15.9
Crude fibre	5.7	5.8	6.0
Calcium	0.91	0.91	0.91
Phosphorus	0.54	0.54	0.54
Lysine	1.46	1.30	1.15
Methionine	0.06	0.05	0.04
DE, MJ/kg	13.6	13.4	13.3

¹CON = control (liquid fermented control with and without enzyme); LLFPH = low inclusion of liquid fermented potato hash with and without enzyme; HLFPH = high inclusion of liquid fermented potato hash with and without enzyme.

²Provided the following per kg of diet: 6,500 IU vitamin A, 1,200 IU vitamin D3, 40 IU vitamin E, 2 mg vitamin K3, 1–5 mg vitamin B1, 4.5 mg vitamin B2, 0.03 mg vitamin B12, 2.5 mg vitamin B6, 25 mg niacin, 12 mg calcium pantothenate, 190.5 mg choline, 0.6 mg folic acid, 0.05 mg biotin, 40 mg manganese, 100 mg zinc, 125 mg copper, 1 mg iodine, 100 mg ferrous, and 0.3 mg selenium

volatiles using the equation of Porter & Murray (2001). Dry samples were ground through a 1 mm screen (Wiley mill, Standard Model 3, Arthur H. Thomas Co., Philadelphia, PA, USA) for chemical analyses.

The neutral detergent fibre (NDF) and acid detergent fibre (ADF) contents were determined following the procedures of Van Soest et al. (1991) using ANKOM Fibre Analyser (Ankom, Macedon, NY, USA). Separate samples were used for ADF and aNDF analyses and both included residual ash. The NDF content was determined without a heat stable amylase. Crude protein (ID 968.06), ash (ID 942.05) and EE (ID 963.15) were determined according to procedures of AOAC (1990). The GE was determined with bomb calorimetry (MS-1000 modular calorimeter, Energy Instrumentation, Centurion, South Africa).

3.2.2.4 Determination of water holding capacity

Water holding capacities of feed were determined according to Whittemore *et al.* (2003). Briefly, 0.5 g of each feed ingredient was placed into a 50 ml centrifuge tube and 25 ml distilled water was also added. Tubes were securely sealed and shaken intermittently for 24 hours and centrifuged at 6000 $\times g$ for 15 min at 20 °C. The supernatant was discarded and fresh weight of the sample was determined. After freeze-drying, the weights of the fluid retained were calculated from the difference between fresh sample and dried sample. The weight of the fluid retained was divided by the weight of the dried sample to determine WHC, which was expressed in g water/g of dry material.

3.2.2.5 Determination of swelling capacity

Swelling capacity was measured based on the modified bed volume technique as described by Canibe and Bach Knudsen (2002). Experimental diets samples (2 g) were weighed into 15 ml measuring plastic tubes, a solution of 9g/ l NaCl containing 0.2g/ l NaN₃ was added to a final volume of 10 ml, where samples were incubated at 39°C in a water shaking bath overnight. After 16h, the shaker was stopped and samples were left in the water for 1 h before being taken out to measure the volume occupied by the fibre. Results were expressed as ml of swollen sample per gram of dry residue. All samples were analysed in triplicate.

3.2.2.6 Determination of bulk density

The densities of the feed, defined as the degree of consistency measured by the quantity of mass per unit volume were measured according to the water displacement method, described by Kyriazakis and Emmans (1995). The method is based on the *Archimedes Principle* of determining the volume of a known mass of feed ingredient. Briefly, 50g of feed were weighed and placed into a 250 ml volumetric flask. First 100 ml distilled water was gently added into the flask and the content was allowed to equilibrate for 15 min. The flask was gently tapped for 10 to 12 times to pack it and additional 50 ml distilled water was added. After allowing 15 min to equilibrate, a measured amount of water was added using a burette to bring to volume. The flask was shaken frequently to minimise the displacement of water by air. The total amount of water contained in the flask was subtracted from 250 ml. To calculate the density, unit mass of the feed ingredient was expressed per unit volume of water displaced (g/ml).

3.2.2.7 Statistical analyses

Chemical composition data was analysed using the general linear model (GLM) procedures of SAS (2008) to determine differences in the chemical composition and physical properties of liquid fermented potato hash, respectively. Separation of least square means was done using the probability of difference (PDIFF) procedure (SAS, 2008). Pearson's correlation coefficients among physicochemical properties of liquid fermented potato hash were estimated using the PROC CORR (SAS, 2008). Pearson's correlation coefficients were also used to determine relationships between calculated and analysed bulk density and WHC of the commercial feed. The model used was:

$Y_{ijk} = \mu + F_i + L_j + (F \times L)_{ij} + E_{ijk}$, Where:

Y_{ijk} – is the response variable

μ - is the overall mean common to all observations

F_i – is the effect of enzyme

L_j – is the effect of enzyme and inclusion level

$(F \times L)_{ij}$ – is the interaction between enzyme and inclusion level

E_{ijk} – is the residual error

3.3 Results

Correlation coefficients among chemical composition and physicochemical properties of liquid fermented potato hash with or without enzymes are shown in Table 3.2. The EE, WHC, SC, Density, NDF and ADF was negatively correlated to DM ($P < 0.05$) and Ash ($P < 0.01$) of liquid fermented potato hash. The bulk density was negatively

correlated ($P < 0.05$) to CP and NDF. The ash content was negatively correlated with CF, NDF and NDF ($P < 0.01$), GE ($P < 0.05$), but positively correlated ($P < 0.05$) with WHC. Table 3.3 shows the chemical composition and physicochemical properties of diets containing different inclusion levels of liquid fermented potato hash treated with or without enzyme. The control diet had a higher ($P < 0.05$) dry matter and ash concentrations compared to diets containing different inclusion levels of liquid fermented diets.

The LLFPH+E and LLFPH had a higher ($P < 0.05$) dry matter and ash concentrations compared to HLFPH+E and HLFPH diets. Also, liquid fermented diets treated with enzyme had a higher dry matter and ash concentrations compared to diets without enzyme. There was no difference ($P > 0.05$) between liquid fermented diets treated with or without enzyme on gross energy and ether extract concentrations. The control, LFC+E and LFC diets had a greater ($P < 0.05$) crude protein compared to liquid fermented potato hash diets. However, LLFPH+E and LLFPH diets had a higher ($P < 0.05$) crude protein than HLFPH+E and HLFPH diets. However, there were no differences ($P > 0.05$) in diet \times enzyme interactions on gross energy, ether extract and crude protein. There were greater ($P < 0.05$) NDF, ADF and swelling capacity in HLFPH+E and HLFPH diets as compared to LLFPH+E and LLFPH diets.

Table 3.2: Correlation coefficients among chemical composition and physicochemical properties of fermented liquid feed potato hash

	¹ Ash	GE	EE	CP	WHC	SC	Density	NDF	ADF
DM	0.69**	-0.57*	-0.29 ^{NS}	-0.38 ^{NS}	-0.48 ^{NS}	-0.58*	-0.38 ^{NS}	-0.43 ^{NS}	-0.49 ^{NS}
Ash		-0.51 ^{NS}	-0.68**	-0.82**	-0.51 ^{NS}	-0.84**	-0.07 ^{NS}	-0.86**	-0.79**
GE			0.29 ^{NS}	0.31 ^{NS}	0.32 ^{NS}	0.47 ^{NS}	0.04 ^{NS}	0.38 ^{NS}	0.39 ^{NS}
EE				0.80**	0.59*	0.66**	0.03 ^{NS}	0.79**	0.79**
CP					0.65*	0.87***	-0.01 ^{NS}	0.98***	0.92***
WHC						0.56*	0.30 ^{NS}	0.56*	0.59*
SC							-0.38 ^{NS}	0.93***	0.97***
Density								-0.01 ^{NS}	-0.04 ^{NS}
NDF									0.95***

Significance level: NS = $P > 0.05$; * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$.

¹DM = dry matter (g/kg); CP=crude protein (g/kg DM); EE=ether extract (g/kg DM); Ash (g/kg DM); NDF = neutral detergent fibre (g/kg DM); ADF=acid detergent fibre (g/kg DM); GE= Gross energy (MJ/kg DM); WHC = water holding capacity (g water/g DM); Density = Bulk density (g DM/ml). SC = swelling capacity

Table 3.3: Chemical composition (g/kg DM feed) and physicochemical properties of varying levels of liquid fermented potato hash diet with or without exogenous enzyme (n=3)

Composition ²	Treatments								P		
	Control	LFC+E	LFC	LLFPH+E	LLFPH	HLFPH+E	HLFPH	SEM	Diet	Enzyme	Diet*Enzyme
Dry matter g/kg DM	904.7 ^a	310.1 ^b	306.1 ^b	294.5 ^{bc}	284.7 ^c	260.4 ^d	256.3 ^d	0.189	<.0001	<.0001	<.0001
Ash g/kg DM	62.0 ^a	58.5 ^b	57.6 ^b	53.6 ^{bc}	51.1 ^{bc}	41.1 ^c	40.6 ^c	0.209	<.0001	<.0001	<.0001
Gross energy (MJ/kg)	18.1	18.1	18.1	17.9	17.9	17.9	17.8	0.656	0.326	0.605	0.728
Ether extract g/kg DM	49.0	49.5	49.5	48.5	44.5	38.9	37.5	4.14	0.048	0.688	0.679
Crude protein g/kg DM	194.0 ^a	191.0 ^a	192.0 ^a	193.5 ^a	186.5 ^b	181.0 ^{bc}	168.0 ^c	1.13	<.0001	0.171	0.495
Physicochemical properties											
WHC (g W/ g DM)	3.8 ^a	2.2 ^b	2.2 ^b	1.7 ^c	1.5 ^c	1.3 ^d	1.2 ^d	0.415	0.028	0.068	0.105
SC (ml/ g)	2.1 ^c	2.1 ^c	2.2 ^c	2.7 ^b	2.7 ^b	3.1 ^a	3.1 ^a	0.082	<.0001	0.359	0.076
Density(g DM/ ml)	1.4	1.5	1.5	1.5	1.6	1.6	1.6	1.69	0.445	0.812	0.257
NDF (g/ kg DM)	132.1 ^d	130.0 ^d	132.1 ^d	202.2 ^c	244.8 ^b	418.1 ^a	465.9 ^a	1.99	<.0001	<.0001	<.0001
ADF (g/ kg DM)	39.1 ^e	36.2 ^e	38.9 ^e	71.3 ^d	80.1 ^c	98.9 ^{ab}	106.85 ^a	0.085	<.0001	0.002	0.020

^{abc} Values with different superscripts within a row are different (P > 0.0001); ¹CON – Control diet; LFC – Liquid fermented control diet, LFCE- Liquid fermented control with enzyme diet, LLFPHE- Low liquid fermented potato hash with enzyme diet, LLFPH- Low liquid fermented potato hash without enzyme diet, HLFPH- High liquid fermented potato hash with enzyme diet, HLFPH- High liquid fermented potato hash without enzyme diet. ²NDF = neutral detergent fibre; ADF = acid detergent fibre; WHC = water holding capacity; SC = Swelling capacity; Bulk density.

The liquid fermented diets treated with enzyme had a lower ($P < 0.05$) NDF and ADF concentrations compared to diets without enzyme. The control diet had a higher ($P < 0.05$) water holding capacity than liquid fermented diets. There was no ($P > 0.05$) difference between diets on bulk density. In addition, there was no differences ($P > 0.05$) due to diet \times enzyme interactions on water holding capacity, swelling capacity and bulk density.

3.4 Discussion

The extent of fermentation of locally available fibrous feed ingredients in pigs is poorly understood. The main aim for fermenting liquid by-products is to maintain the original quality of the preserved by product as much as possible (Wilkinson and Davies, 2012). As a result, additives have been used to direct the fermentation process towards the production of lactic acid as the main fermentation product. Fermented liquid diets normally contain 20 to 30% dry matter content (de Lange *et al.*, 2006). The DM content of fermented liquid feed has a strong influence on the rate and extent of the resulting fermentation. In this study, DM content of fermented liquid potato hash with or without enzyme was less than 300 g/kg. One of the most important aspects of using liquid fermented feeding successfully is to ensure that the proper ratio of water: dry matter content and frequency of feeding is achieved for the specific production phases where it is used (de Lange *et al.*, 2006).

In the current study, water and feed were mixed at a ratio of 2:1 for 8hr. Results of the current study are consistent with those by (Brooks *et al.*, 2003 and Lyberg *et al.* 2008) where water and feed ratio was 2:1. The higher CP content in low and high liquid fermented potato hash with enzyme compared to low and high liquid fermented potato

without enzyme are consistent with the findings of Dean *et al.* (2005) and Rodriguez *et al.* (2001). The reduced fibre content with low and high liquid fermented potato hash with enzyme compared to low and high liquid fermented potato without enzyme are consistent with previous observations on enzyme-treated potato hash silage (Nkosi *et al.*, 2015). The reduction in fibre content (NDF and ADF) with low and high liquid fermented potato hash with enzyme treatment could be attributed to partial hydrolysis of hemicelluloses (Muck and Kung, 1997). This supports other studies that reported reduced fibre content with enzyme (Ozkose *et al.*, 2009; Nkosi and Meeske, 2010; Nkosi *et al.*, 2011).

Characterisation of physicochemical properties, such as the fibre content, bulk density and WHC of feed ingredients is vital in designing formulation strategies to ensure that feed bulk does not constrain intake of sufficient nutrients required for growth. Low WHC values observed for liquid fermented potato hash diets with or without enzyme could be explained by the vast pool of charged groups within their matrices which decreases their hydrophilic nature thereby increasing their ability to bind water. The hypothesis is supported by the considerably lower ash content of these feed ingredients compared to other feedstuffs with high water holding capacities.

The observed low WHC capacities of liquid fermented potato hash could decrease in feed intake when fed to growing pigs. High protein diets increase the need for water intake to cater for excretion of surplus dietary mineral intake and catabolised nitrogenous compounds from protein (Pfeiffer *et al.*, 1995). The WHC was highest for low fermented liquid potato hash with or without enzyme compared high liquid

fermented potato hash with or without enzyme. The difference in WHC of the fibrous sources can be attributed to the fact that exposure of hydrophilic binding sites within the fibre matrix differ with fibre types principally because of the differences in the polysaccharide building blocks forming the structure of those feedstuffs (Elleuch *et al.*, 2011). Similarities in WHC noticed could be explained by grinding which uniformly exposes those hydrophilic sites, thereby consolidating the water binding capacity of the fibre. The similarities can also be due to the congruency of the non-starch polysaccharide types of the fibrous ingredients, especially those of potato hash.

Thus, studies to characterize the nature of the polysaccharide composition should be considered if a valid explanation of the effects these fibrous feedstuffs have on feed bulk and, consequently, feed consumption in growing pigs, is to be achieved. Inclusion of high fermented liquid potato hash diets increased swelling capacity compared to low fermented liquid potato hash diets. Swelling capacity of feed forms the first part of the solubilisation process whereby the incoming water spreads the macromolecules of dietary fibre components until they fully extend and get disseminated (Bach Knudsen, 2001; Knudsen, 2011). High fibre content is expected to increase the volume and the extent to which the fibre component expands (Bach Knudsen, 2001). Okine *et al.* (2005) described by-products from the food industry as soluble fibre source. Soluble fibres are generally highly fermentable due to their physiological effects to increase the surface area of the substrate for easy colonisation and effective degradation (Bach Knudsen, 2001). The decrease in SC of the low liquid fermented potato hash diets could have been due to increased susceptibility of microbial action (Canibe and Bach Knudsen, 2002).

3.5 Conclusions

The varying levels of fermented liquid potato hash did not negatively affect the physicochemical properties and nutrient composition of the diets. The use of exogenous enzyme during fermentation reduced the fibre fractions of the fermented liquid potato hash even though it was at the expense of energy content of the diets. The reduction of fibre content could enhance intake and digestibility of nutrients when offered to pigs as a component of a balanced diet. Further work is needed to investigate the effects of fermented liquid potato hash diets on microbial characteristics, digestibility of nutrients and feed intake.

3.6 References

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Chapter 4

Microbial characteristics of varying levels of fermented liquid potato hash feed with or without exogenous enzyme during fermentation

Abstract

The objective of the study was to determine microbial characteristics in varying levels of fermented liquid potato hash in diets treated with or without an exogenous enzyme. Six experimental diets were formulated to be isoenergetic and isonitrogenous containing different amounts of Liquid Fermented Potato Hash (LFPH): 0, 200, 400 g kg⁻¹ DM treated with or without enzyme to provide 14 MJ/kg digestible energy (DE), 180 g crude protein (CP)/kg and 11.6 g lysine /kg which. Feed and water were mixed in the ratio 1:2, and fermented for 8 hrs. Diets were stored in a closed 100 L drum under agitation at 25°C. Measurements of pH were taken in triplicate for three weeks. A pH sample was taken after 0, 8 and 24 hrs of fermentation. The lactic acid (LA) and water soluble carbohydrates (WSC) measurements were taken from three different batches in triplicate. There were no differences ($P > 0.05$) across the three batches on 0 hr between different inclusion levels of liquid fermented potato hash with or without enzyme on pH. However, pH decreased rapidly from 8 to 24 hrs across the three batches. The liquid fermented control with enzyme (LFC+E), low liquid fermented potato hash with enzyme (LLFPH+E) and high Liquid fermented potato hash with enzyme (HLFPH+E) had lower pH values at 8 and 24 hrs ($P < 0.05$) compared to liquid fermented control (LFC), low liquid fermented potato hash (LLFPH) and high liquid fermented potato hash (HLFPH). Enzyme treated liquid fermented potato hash diets reduced pH and increased WSC rapidly than liquid fermented potato hash without enzyme. The number of lactic acid bacteria in the LFC+E, LLFPH+E and HLFPH+E

was increased from <3.0 log cfu/g to 8.96, log cfu/g 9.04 log cfu/g and 8.94 log cfu/g, respectively, compared to LFC, LLFPH and HLFPH <3.0 log cfu/g to 6.3 log cfu/g, 6.05 log cfu/g and 6.01 log cfu/g respectively during the 8 h of fermentation. *Enterobacteriaceae* counts bacteria in the LFC+E, LLFPH+E and HLFPH+E was decreased form 6.0 log cfu/g to 4.2, log cfu/g 4.3 log cfu/g and 4.6 log cfu/g, respectively, compared to LFC, LLFPH and HLFPH 6.0 log cfu/g sample to 5.0 log cfu/g, 4.6 log cfu/g and 4.6 log cfu/g, respectively, during the 8 h of fermentation. Addition of enzyme to liquid fermented potato hash increased LA production and decreased pH. Further studies are needed in which identification of the microbial population in liquid fermented potato hash to strain level and quantification of metabolites from carbohydrate and amino acid fermentation. With this knowledge, strategies that promote beneficial strains and that impede the proliferation of undesired strains resulting in FLF with optimal microbial characteristics will be much easier to establish.

Keywords: Fermented liquid feed, Microbial quality, Potato hash, Enzyme

4.1 Introduction

The use of fermented liquid feed (FLF) in pig production is widespread (Scholten *et al.*, 1999; Brooks *et al.*, 2003) and interest for it has increased due to the ban of antibiotic growth promoters in pig feed. Also, the increasing popularity of using fermented liquid feeding systems is being driven by a tremendous increase in availability and low cost of liquid by-products from the food industry (Van Winsen *et al.*, 2000; Beal *et al.*, 2002; Canibe and Jensen, 2003). By-products of industrial potato processing are a potential feed resource which could be used as liquid feed in pig diets. Potato hash, a potato by-product derived from the production of chips and snacks (Schieber *et al.*, 2001), produced at Simba (Pty) Ltd (Isando, Gauteng Province) at the rate of 50 tons per day is one such potential ingredient to be used as liquid feed. This by-product contains a dry matter (DM) of 150 g/kg (Nkosi, 2009). The low DM concentration in potato hash is attributed to the use of large amounts of water in washing and processing, some of which remains in the hash. By-products with high moisture content tend to deteriorate or become moldy very quickly (Kajikawa, 1996). Such by-products can be fed as liquid fermented feed to pigs.

Fermentation of animal feed confers protection against pathogens that the raw materials might harbour or that might contaminate the feed during storage (Adams and Nicolaides, 1997; van Winsen *et al.*, 2000; Beal *et al.*, 2002; Canibe and Jensen, 2003). Successful fermentations of liquid feed can be obtained by mixing fresh feed and water with material from a previous successful fermentation, which acts as inoculum for the new mixture (Jensen and Mikkelsen, 1998; Scholten, 2001; Canibe and Jensen, 2003). The final product is be of good microbial quality and biosafe only if the amount of inoculum, the temperature at which the mixture is maintained, and the

interval between addition of fresh feed and water to the next addition (turn-over) are appropriate (Jensen and Mikkelsen, 1998; Scholten, 2001; Beal *et al.*, 2002). Characteristics of FLF prepared when combining the appropriate amount of inoculum, temperature and turn-over are: low pH, high numbers of lactic acid bacteria, low numbers of *Enterobacteriaceae*, high concentrations of lactic acid, and, to a lower extent, of acetic acid (Jensen and Mikkelsen, 1998; Canibe and Jensen, 2003).

Various strategies, like addition of starter cultures, exogenous enzymes and addition of organic acids, have been used to avoid or attenuate the development of high levels of *Enterobacteriaceae* in the liquid feed (Geary *et al.*, 1999; Canibe *et al.*, 2001). The objective of the present study was to determine the microbial characteristics of varying levels liquid fermented potato hash diet treated with or without enzyme.

4.2 Materials and methods

4.2.1 Feed, experimental design and experimental procedure

Six experimental diets were formulated to be isoenergetic and isonitrogenous containing different amounts of Liquid Fermented Potato Hash (LFPH): 0, 200, 400 g kg⁻¹ DM treated with or without an exogenous enzyme. Diets were formulated to provide 14 MJ/kg digestible energy (DE), 180 g crude protein (CP)/kg and 11.6 g lysine /kg which meet and exceed the requirements of growing pigs (NRC, 1998). The six diets were; fermented control diet (LFC), diet containing 200 g potato hash.kg⁻¹ diet, as fed (LLFPH), diet containing 400 g potato hash.kg⁻¹ diet, as fed (HLFPH), fermented control diet treated with enzyme (LFCE), diet containing 200 g potato hash.kg⁻¹ diet treated with enzyme, as fed (LFPHE), diet containing 400 g potato hash.kg⁻¹ diet treated with enzyme, as fed (HFPHE).

4.2.2. Fermentation of diets and experimental design

The back-slopping fermentation approach was followed as described by Plumed-Ferrer and Von Wright, (2009). Fermented liquid diets were prepared by mixing diet with water, at a ratio of 1:2 for 8 hrs. An exogenous xylanase enzyme (Natugrain TS L®), was added at (0.5 g/kg-1) before fermentation of diets. Fermented diets were stored in a closed 100 L drum under agitation at 25 °C. After 8 hrs, 50 % of the content was taken out and replaced in the drum with an equal amount of fresh feed and water. A sample from different batches was taken at 0, 8 and 24 hrs of fermentation to determine the pH of the diets.

4.2.3 Analytical methods

Dry matter (DM) content in triplicate was determined by oven-drying the samples at 65 °C for 48 hrs. Total anaerobic bacteria, *Lactobacillus* sp, *Escherichia coli*, *Salmonella* sp, *Clostridium perfringens* were determined in triplicate according to the method of Jensen *et al.* (1995). For microbiological enumeration, liquid feed samples (10 g) were transferred into flasks containing 90 mL of peptone water containing 10.0 g Bacto peptone (Merck 1.07213, Darmstadt, Germany)/L and 1.0 g Tween 80/L. The suspension was then transferred to a plastic bag and homogenised in a stomacher blender (Interscience, St. Nom, France) for 2 min. Then, 10-fold dilutions were prepared in peptone water by the technique of Miller and Wolin (1974), and samples (0.1 mL) were plated on selective media.

Concentration of lactic acid was assayed by the method of Jensen *et al.* (1995) on de Man, Rogosa and Sharp agar (Merck 1.10660.0500) following anaerobic incubation at 20 °C for 3 days. *Enterobacteriaceae* were enumerated on McConkey agar (Merck

1.05465.0500) following aerobic incubation at 37 °C for 1 day. Yeasts and moulds were enumerated on malt chloramphenicol/chlortetracycline MCA agar (10 g glucose [Merck 1.08337.1000]/L; 3 g malt extract [Merck 1.05397]/L; 3 g yeast extract [Merck 1.03753]/L; 5 g Bacto peptone [Merck 1.07224]/L; 50 mg chlortetracycline + 50 mg chloramphenicol [SR0177E, Oxoid Ltd., Basingstoke, Hampshire, UK]/L; 15 g agar [Merck 1.01614]/L following aerobic incubation at 20 °C for 3 days. The pH was determined immediately with a pH meter (Thermo Orion Model 525, Thermo Fisher Scientific, Waltham, MA, USA). The pH was taken at 0, 8 and 24 hrs for three different weeks in triplicates. The WSC fraction was determined by the phenol-sulphuric acid method of Dubois *et al.* (1956). LA was determined by the colorimetric method of Pryce (1969). Samples were taken in three different batches after 8 hrs of incubation.

4.2.4 Statistical analyses

Data on effects of treatments on fermentation were analysed in a completely randomized design by ANOVA using Genstat (2011). Pearson's correlation coefficients among microbial populations fermented liquid potato hash were estimated using the PROC CORR (SAS, 2008). Differences among treatment means were compared with least significant difference (LSD) and significance was declared at the 0.05% probability level. Data was fitted to the model: $Y_{ijk} = \mu + E_i + B_j + (E \times B)_{ij} + \varepsilon(ijk)$,

Where

- μ = overall mean

- E_i = effect of the enzyme

- B_j = effect of the jth inclusion level (fermentation)

-(EB)_{ij} = effect of interaction between the enzyme and jth inclusion level (fermentation)

- ϵ_{ijk} = residual error

The detection level was 6 log cfu/g for *Enterobacteriaceae* and 3 log cfu/g for lactic acid bacteria and yeasts.

4.3 Results

Data on change in pH of liquid fermented potato hash with or without enzyme are shown in Table 4.1. There were no differences ($P > 0.05$) across the three batches on 0 hr between different inclusion levels of fermented liquid potato hash with or without enzyme on pH. However, pH decreased rapidly from 8 to 24 hrs across the three batches. The LFC+E, LLFPH+E and HLFPH+E had lower pH values at 8 and 24 hrs ($P < 0.05$) compared to LFC, LLFPH and HLFPH. The microbial populations of varying levels of liquid fermented potato hash are shown in Table 4.2. The number of lactic acid bacteria in the LFC+E, LLFPH+E and HLFPH+E was increased compared to LFC, LLFPH and HLFPH during the 8 hrs of fermentation. Enterobacteriaceae counts bacteria in the LFC+E, LLFPH+E and HLFPH+E was decreased compared to LFC, LLFPH and HLFPH during the 8 hrs of fermentation. The yeast counts were also low in the LFC+E, LLFPH+E and HLFPH+E compared to LFC, LLFPH and HLFPH. There was no interaction between diets and enzyme on LA and WSC concentrations between the treatments as shown in Table 4.3. The LLFPH+E and HLFPH+E reduced WSC and increased LA concentration compared to LFC+E, LFC, LLFPH and HLFPH. Table 4.4 shows correlation coefficients among microbial populations fermented liquid potato hash. The *Enterobacteriaceae* was negatively correlated to Yeast and Moulds

($P < 0.05$) of the fermented liquid potato hash. The LAB was positively correlated ($P < 0.05$) to LA and WSC.

Table 4.1: Least square means of pH of different inclusion levels of liquid fermented potato hash with or without enzyme (n=3)

		Treatments									
	(hrs)	LFC	LFC+E	LLFPH+E	LLFPH	HLFPH+E	HLFPH	SD	Diet	Enzyme	Diet*Enzyme
Batch 1	0	5.04	5.01	5.03	5.05	5.05	5.08	0.85	0.8897	0.7759	0.8553
	8	3.61 ^b	3.58 ^a	3.55 ^a	3.69 ^c	3.57 ^a	3.70 ^c	0.32	<.0001	<.0001	0.0046
	24	3.58 ^b	3.54 ^a	3.53 ^a	3.60 ^b	3.53 ^a	3.61 ^b	0.91	<.0001	<.0001	0.0285
Batch 2	0	5.03	5.06	5.02	5.01	5.05	5.05	0.28	0.6524	0.6289	0.6629
	8	3.59 ^b	3.55 ^a	3.54 ^a	3.59 ^b	3.57 ^a	3.59 ^b	0.41	<.0001	<.0001	0.0183
	24	3.57 ^a	3.62 ^b	3.54 ^a	3.63 ^b	3.55 ^a	3.62 ^b	0.25	<.0001	<.0001	0.0126
Batch 3	0	5.03	5.06	5.02	5.01	5.05	5.05	0.47	0.6263	0.6322	0.3107
	8	3.60 ^b	3.57 ^a	3.56 ^a	3.62 ^b	3.57 ^a	3.62 ^b	0.54	<.0001	<.0001	0.0005
	24	3.60 ^b	3.55 ^a	3.56 ^a	3.61 ^b	3.56 ^a	3.62 ^b	0.51	<.0001	<.0001	0.0002

^{a,b} Means in the same column within the same section with different superscripts differ significantly (P<0.0001). LFC- Liquid fermented control; LFC+E- Liquid fermented control with enzyme; LLFPH+E- Liquid fermented potato hash with enzyme; LLFPH- Liquid fermented potato hash without enzyme; HLFPH+E- Liquid fermented potato hash with enzyme; HLFPH- Liquid fermented potato hash without enzyme

Table 4.2: Microbial populations (log cfu/g sample) in varying levels of liquid potato hash diet during fermented a (n=3)

Parameters ^c	Treatments ^a						Sig.			
	LFC	LFC+E	LLFPH+E	LLFPH	HLFPH+E	HLFPH	SD	Diet	Enzyme	Diet * Enzyme
LAB	6.3 ^b	8.9 ^a	9.0 ^a	6.1 ^b	8.9 ^a	6.0 ^b	0.43	**	*	**
Enterobacteriaceae	5.0 ^b	4.2 ^a	4.3 ^a	5.5 ^b	4.6 ^a	5.5 ^b	0.56	*	*	NS
Yeast and Moulds	5.6 ^a	4.5 ^b	4.4 ^b	5.6 ^a	4.6 ^b	5.6 ^a	0.29	**	**	NS
<i>E.coli</i>	ND	ND	ND	ND	ND	ND	ND	NS	NS	NS

^{a, b} Values are means, Sig. = Significance level. * $P < 0.05$; *** $P < 0.001$, NS – Not significant, LFC – Liquid fermented control diet, LFCE- Liquid fermented control with enzyme diet, ^aLLFPHE- Low liquid fermented potato hash with enzyme diet, LLFPH- Low liquid fermented potato hash without enzyme diet, HLFPH- High liquid fermented potato hash with enzyme diet, HLFPH- High liquid fermented potato hash without enzyme diet. ^b Detection level was 6 log cfu/g for Enterobacteriaceae, <3 log cfu/g for lactic acid bacteria (LAB) and yeasts for all diets. *E. coli* was not detected.

Table 4.3: Lactic acid and water soluble carbohydrates (g/kg DM) of liquid fermented potato diets with or without enzyme (n=3)

		Diet ¹							P		
	Parameters ²	LFC	LFC+E	LLFPH+E	LLFPH	HLFPH+E	HLFPH	SEM	Diet	Enzyme	D*E
Batch1	LA	66.01 ^d	71.6 ^{bc}	87.2 ^a	79.4 ^b	84.9 ^a	74.9 ^b	3.57	**	**	NS
	WSC	61.3 ^c	69.3 ^b	74.9 ^a	67.4 ^b	75.4 ^a	64.3 ^b	6.27	**	**	NS
Batch1	LA	65.3 ^d	71.8 ^{bc}	84.1 ^a	75.4 ^b	83.7 ^a	73.3 ^b	3.46	**	**	NS
	WSC	60.6 ^c	68.3 ^b	73.4 ^a	63.6 ^b	74.9 ^a	63.7 ^b	4.43	**	**	NS
Batch1	LA	67.4 ^d	72.7 ^{bc}	84.8 ^a	76.9 ^b	83.9 ^a	74.7 ^b	3.54	**	**	NS
	WSC	64.0 ^c	67.7 ^b	74.9 ^a	63.9 ^b	75.1 ^a	63.4 ^b	4.43	**	**	NS

^{a-f} Values in the same column within the same section with different superscripts differ significantly (P<0.01). NS= not significant; **P<0.001. ¹ LFC – Liquid fermented control diet, LFCE- Liquid fermented control with enzyme diet, LLFPHE- Low liquid fermented potato hash with enzyme diet, LLFPH- Low liquid fermented potato hash without enzyme diet, HLFPH- High liquid fermented potato hash with enzyme diet, HLFPH- High liquid fermented potato hash without enzyme diet. ² LA= lactic acid, WSC= water soluble carbohydrates

Table 4.4: Correlation coefficients among microbial populations fermented liquid potato hash

	Enterobacteriaceae	Yeast and Moulds	LA	WSC
LAB	0.67*	0.71*	0.86**	0.81**
Enterobacteriaceae		-0.67**	0.59*	0.60*
Yeast and Moulds			0.47 ^{NS}	0.44 ^{NS}
LA				0.83*

Significance level: NS = $P > 0.05$; * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$

Detection level was 6 log cfu/g for Enterobacteriaceae, <3 log cfu/g for lactic acid bacteria (LAB) and yeasts, LA= lactic acid, WSC= water soluble carbohydrates

4.4 Discussion

Food fermentation is one of the oldest ways of food processing and preservation. The use of liquid feeds in animals has created an opportunity for recycling of liquid by-products from the human food industry especially in pig nutrition (Scholten *et al.*, 1999; Brooks *et al.*, 2003). Brooks *et al.* (2001), stated that liquid-feeding systems can easily become contaminated. They further observed that the development of computerised liquid-feeding systems capable of feeding pigs *ad libitum* has rekindled interest in the possibility of liquid feeding for pigs. This, in addition to recent developments in the use of additives such as enzyme in the accelerated fermentation of feed substrates for animal feeding as well as reducing the possibility of contamination by *enteropathogens* (Beal *et al.*, 2002; Beal *et al.*, 2005), has provided a good basis for improvement in pig nutrition. In the current study, fermented liquid potato hash diets treated with enzymes had a low *enteropathogens* which are consistent with those studies.

Lactic acid (LA) is the strongest of all liquid fermented diets acids and its presence will drop pH more effectively than VFAs (McDonald *et al.*, 2010). This is consistent with the current study which recorded a higher LA and low pH on fermented liquid potato with enzymes. It is well-documented that one of the most important factors affecting fermented liquid diet quality is the rate of decrease in pH of material being preserved (Moran *et al.*, 2006). A pH range of 3.8–4.2 (Geary *et al.*, 1996), Christensen *et al.* (2007) (3.6-4.2), Scholten *et al.* (1999) (3.5-4.5) and Moran *et al.* (2006) (less than 3.8) is considered beneficial for liquid fermented diets.

The pH for the current study was less than 3.6, which is consistent with those studies and it indicates that fermented liquid potato hash diets was well-preserved. The

reduction of pH on enzyme treated diets are in agreement with previous studies (Canibe *et al.*, 2001; Scholten *et al.*, 2001; Moran, 2001; Beal *et al.*, 2002). The low pH and greater lactic acid (LA) in the enzyme treated fermented liquid diets compared to control was due an increase in fermentable carbohydrates by hydrolysis of cell wall which causes fermentation by LAB. The synergistic effect of a high lactic acid concentration and low pH is believed to act in concert to give fermented feeds their antimicrobial activity. This enables them to withstand contamination by pathogens like *Salmonella* spp (Geary *et al.*, 1996; van Winsen *et al.*, 2001; Beal *et al.*, 2002; van Winsen *et al.*, 2002), *Campylobacter* spp (Heres *et al.*, 2003), and coliforms (Russell *et al.*, 1996). High lactic acid concentration and low pH in the feed are desired because this combination prevents the proliferation of spoilage organisms and foodborne pathogens (Nout *et al.*, 1989; Russell and Diez-Gonzales, 1998; van Winsen *et al.*, 2001a). Moreover, feeding liquid feed with high concentration of lactic acid and low pH can result in high lactic acid concentration and low pH in the stomach of the animals, which prevents the proliferation of pathogens along the GI-tract (e.g. *Enterobacteriaceae* such as coliforms and *Salmonella*) (van Winsen *et al.*, 2001b; Canibe and Jensen, 2003; Højberg *et al.*, 2003; Hong *et al.*, 2009) and can improve dietary protein hydrolysis (Kemme *et al.*, 1999; Lyberg *et al.*, 2006).

The increase of pH during the first hours of incubation was probably due to the acid binding capacity of the dietary ingredients (Scholten *et al.*, 2001a). All diets showed a drop in pH for 8 to 24 h of fermentation. As in the study of Canibe *et al.* (2001), lactic acid bacteria count in the LLFPHE and HLPHE diet increased during fermentation to reach values of ~9 log cfu/g. This agrees with Carlson and Poulsen, (2003) who reported high levels of LAB when wheat or barley were fermented. Also, Canibe *et al.*

(2001) showed that 1.0 g formic acid/kg liquid feed allowed proliferation of lactic acid bacteria while impeding the growth of *Enterobacteriaceae*.

The decreasing counts of *Enterobacteriaceae* in the liquid fermented potato diets followed a pattern previously observed by Canibe *et al.* (2001). Hence, addition of enzyme to liquid fermented feed was an effective strategy to avoid a blooming of *Enterobacteriaceae* during the initial hours of liquid feed fermentation. In the LLFPHE and HLPHE diet, it was the low pH and high lactic acid level that reduced the *Enterobacteriaceae* (Jensen and Mikkelsen, 1998; van Winsen *et al.*, 2000), and therefore the time needed for lactic acid bacteria to proliferate and produce high amounts of lactic acid corresponded to the interval measured before *Enterobacteriaceae* levels started to decrease. In the present study, the counts of yeasts during fermentation of liquid feed were lower than previously observed (Geary *et al.*, 1999; Demeckov'a *et al.*, 2000).

Water-soluble carbohydrates are regarded as essential substrates for growth of LAB for proper fermentation (McDonald *et al.*, 1991), and low levels may restrict LAB growth. Lunden-Pettersson and Lindgren (1990) recommended an amount of 60–70 g WSC/kg DM for achieving well-preserved diets. Both LLFPH and HLPHE treated with enzyme had greater residual sugar compared to other treatments. This agrees with Nadeau *et al.* (2000) who reported improved sugar content in cellulose treated alfalfa treated with enzyme.

4.5 Conclusions

It can be concluded that diets that were treated with enzymes in this study had a good microbial quality which suggests liquid fermented liquid potato hash was well-preserved. Addition of enzyme to liquid fermented liquid potato hash increased LA production and decreased pH. Further studies are needed in which identification of the microbial population in liquid fermented liquid potato hash to strain level and quantification of metabolites from carbohydrate and amino acid fermentation. With this knowledge, strategies that promote beneficial strains and that impede the proliferation of undesired strains resulting in FLF with optimal microbial characteristics will be much easier to establish. There is need to assess fermented liquid potato hash treated with enzyme on the effects on feed intake.

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Chapter 5

Effect of varying levels of fermented liquid potato hash diet with or without exogenous enzyme on feed intake and growth performance of growing Large White × Landrace crossbred pigs

Abstract

The objective of the current study was to examine the impact of non-fermented feed and different inclusion levels of fermented liquid potato hash feed treated with or without exogenous enzyme on feed intake and growth performance of growing Large White × Landrace crossbred pigs. Forty-two crossbred male pigs (Large White x Landrace) aged 55 days with average body weight of 25.5 ± 3 kg were selected for this study. Pigs were fed *ad libitum* CON (control diet that was not fermented and without potato hash), LFC (fermented liquid control diet), LLPH (diet containing 200 g potato hash.kg⁻¹ diet, as fed), HLFPH (diet containing 400 g potato hash.kg⁻¹diet, as fed), LFCF (fermented liquid control diet treated with an exogenous xylanase enzyme (Natugrain TS L®), LLPFHE (diet containing 200 g potato hash.kg⁻¹ diet treated with an exogenous xylanase enzyme (Natugrain TS L®), as fed), HLPFHE (diet containing 400 g potato hash.kg⁻¹diet treated with an exogenous xylanase enzyme (Natugrain TS L®), in a completely randomized block design. The back-slopping fermentation approach was followed to prepare fermented diets. Diets were formulated to provide 14 MJ/kg digestible energy (DE), 180 g crude protein (CP)/kg and 11.6 g lysine /kg. The LLPFPH+E had greater ($P < 0.05$) final weight (FW), average daily feed intake (ADFI), dry matter intake (DMI), average daily gain (ADG) and feed conversion ratio (FCR) than the LLPFPH, HLPFHE, LFC. There was no difference ($P < 0.05$) between

LLFPHE and control diets. The fermented liquid diets treated with enzyme improved ($P < 0.05$) feed intake of grower pigs. There were diet x enzyme interactions for average daily intake and feed conversion ratio in the growing pigs. The overall performance of pig fed different inclusion levels of fermented liquid potato hash diets treated with or without enzyme did not negatively impact growth performance of grower pigs. Therefore, since the inclusion level of fermented liquid potato hash diets treated with or without enzyme did not affect negatively the growth performance of grower pigs, this implies that different inclusion level of fermented liquid potato hash can be used successfully as pig feed.

Keywords: liquid feed, fermentation, potato hash, feed intake, growth performance, grower pigs

5.1 Introduction

Concepts of non-fermented liquid feed (NFLF) and fermented liquid feed (FLF) have been defined by Canibe and Jensen (2003). The former is as a mixture of feed and water made immediately before feeding. The latter is a mixture of feed, such as a source of agro by-products, or a complete feed and water stored in a tank at a certain temperature and for at least an 8 hr period before feeding to the animal. A characteristic of FLF is a high concentration of lactic acid bacteria, yeasts, and lactic acid, low pH, and low enterobacteria counts. Benefits of liquid feed have been shown by many researchers (Scholten *et al.*, 1999; Scholten, 2001; Canibe and Jensen, 2003; Brooks, 2008), and include an increase in daily feed intake and live weight gain compared with dry feeding (Brooks *et al.*, 1996; Jensen and Mikkeisen 1998). Beneficial influence of FLF feeding compared to dry feed or non-FLF on gastrointestinal health has been reported including reduction in the number of several pathogens in the gastrointestinal-tract (GI-tract) of growing pigs and sows (Demeckova *et al.*, 2002; van Winsen *et al.*, 2002; Canibe and Jensen, 2003; Hong *et al.*, 2009). On the other hand, fermentation can be a means of improving the nutritional value of feed before being offered to the pigs (Carlson and Poulsen, 2003; Lyberg *et al.*, 2006; Shimelis and Rakshit, 2008).

Pigs do not produce enzymes that are able to degrade non-starch polysaccharides (NSP) (Bach Knudsen and Jørgensen, 2001). These substrates are considered as indigestible in the small intestine but in the large intestine a variable fraction of fibre will be fermented by microflora to short-chain fatty acids, and thereby serve as a source of energy for the pig, and also to substantial amounts of gases. In growing pigs, however, the capacity of the microflora to degrade the NSP is less developed than in

older pigs (Graham *et al.*, 1988). Therefore, the lack of enzymatic capacity might be compensated for by supplementation of the diet with exogenous enzymes. It has been reported that exogenous enzymes improve nutrient digestion in pigs (Jones *et al.*, 2010; Kerr and Shurson, 2013). Currently, there is limited data available on the use of exogenous enzymes to reduce fibre levels in potato hash.

In addition, there is limited literature on the effect of fermented liquid potato hash diet on feed intake and growth performance of grower pigs. The lack of such information makes it difficult for stock-feed manufacturers to formulate feeds containing fibrous materials that will allow the optimum utilization of nutrients (Whittemore *et al.*, 2001). The aim of the study was to examine the effects of non-fermented feed and different inclusion levels of fermented liquid potato hash feed diet treated with or without exogenous enzyme, on feed intake and growth performance of Large White × Landrace crossbred pigs.

5.2 Materials and methods

5.2.1 Study site

The study was conducted at the Agricultural Research Council (ARC), Animal Production Institute (API), (ARC-API: Irene, Pretoria). Potato hash (PH) was collected weekly from Simba (336 Andre Greyvenstein road, Isando, Gauteng, South Africa), a local food producing factory in South Africa for production of fermented liquid potato hash diet (LFPH).

5.2.2 Ethical consideration

The experiment was approved by the Agricultural Research Council Animal Ethics committee (Reference Number: APIEC16/005).

5.2.3 Diets

Seven diets were formulated to be isoenergetic and isonitrogenous containing different amounts of Fermented Liquid Potato Hash (LFPH): 0, 200, 400 g kg⁻¹ DM treated with or without enzyme. The diets were formulated to provide 14 MJ/kg digestible energy (DE), 180 g crude protein (CP)/kg and 11.6 g lysine /kg which meet and exceed the requirements of growing pigs (NRC, 1998). Seven dietary treatments were; CON (control diet that was not fermented and without potato hash), fermented control diet, LFPH (diet containing 200 g potato hash.kg⁻¹ diet, as fed), HFPH (diet containing 400 g potato hash.kg⁻¹ diet, as fed), fermented control diet treated with an exogenous xylanase enzyme (Natugrain TS L®), LFPHE (diet containing 200 g potato hash.kg⁻¹ diet treated with an exogenous xylanase enzyme (Natugrain TS L®), as fed), HFPHE (diet containing 400 g potato hash.kg⁻¹ diet treated with an exogenous xylanase enzyme (Natugrain TS L®), as fed).

An exogenous xylanase enzyme (Natugrain TS L®), as fed was added at 0.5 g/kg⁻¹ before fermenting diets. Pigs were adapted to diets for a period of 10 d. The pigs were fed *ad libitum* until they reached an average weight of 60 ± 4 kg. Each of the seven diets were fed to six randomly selected pigs.

5.2.4 Fermentation of diets

The back-slopping fermentation approach was followed as described by Plumed-Ferrer and Von Wright (2009). Fermented liquid diets were prepared by mixing diet with water, at a ratio of 1:2. The fermented diets were stored in a closed 100 L drum under agitation at 25 °C for 8 hrs before being offered to pigs. At each feeding, 50 % of the content was taken out and replaced in the drum with an equal amount of fresh feed. Diets were prepared in quantities sufficient to feed pigs for a week in order to prevent spoilage. Feed was supplied *ad-libitum* and water was made available at all times through drinking nipples. The total mix ration (TMR) was formulated to have 60 % moisture.

5.2.5 Pigs and housing

Forty-two (six pigs per treatment) crossbred male pigs (Large White x Landrace) aged 55 days with average weight of 25.5 ± 3 kg were randomly selected from the ARC-API Irene, pig breeding unit. Pigs were housed individually in 1.54 × 0.8 m pens in environmentally controlled house with the temperature ranging from 22 to 25 °C. Feeders were checked and adjusted twice each day to ensure constant access to fresh feed and minimize any possible wastage.

5.2.6 Measurement of pig performance

During the study, pigs were weighed individually on a weekly basis to determine daily body weight gain. Daily feed offered and weekly orts were recorded. Orts were dried, weighed and discarded daily. Weights of feed refusals and orts were subtracted from the total weight of the feed allocated to determine feed intake for that week. Weight of the feed consumed each week was divided by seven to determine the average daily

feed intake. Feed was supplied *ad libitum* and water was made available at all times through drinking nipples. Mortality and morbidities were noted. Morbidities were diagnosed and the necessary treatments were done.

5.2.7 Statistical analyses

Data for growth performance of grower pigs fed fermented liquid potato hash diets treated with or without enzyme was analysed for effects of treatment using General Linear Model (GLM) procedures of SAS (SAS Inst. Inc., Cary, NC). Pearson's correlation coefficients among performance of grower pigs fed liquid fermented potato hash were estimated using the PROC CORR (SAS, 2008). All data were tested for normality and homogeneity and comparisons were made to the 95 % significance level. The model used was:

$$Y_{ijkl} = \mu + \alpha_i + \beta_j + (\alpha \times \beta)_{ij} + \epsilon_{ijk};$$

where:

Y_{ijkl} is the performance parameter (ADFI, ADG and FCR);

μ is the overall mean response common to all observations;

α_i is the effect of fermentation ($i = \text{F con, F con + E, LPH + E, HPH + E, LPH, HPH}$);

β_j is the effect of enzyme ($j = \text{E}$);

$(\alpha \times \beta)_{ij}$ is the interaction between the enzyme and inclusion level and ϵ_{ijk} is the residual error.

Table 5.1: Growth performance of growing Large White x Landrace (LW x LR) pigs fed diets containing liquid fermented potato hash (n=6)

Parameters ²	Treatments ¹								P		
	CON	LFC	LFC+E	LLFPH+E	LLFPH	HLFPH+E	HLFPH	SEM	Diet	Enzyme	Diet*Enzyme
IW (kg)	24.50	23.07	23.57	22.98	22.33	21.15	22.37	5.61	0.785	0.991	0.903
FW (kg)	77.55 ^a	66.23 ^{bc}	67.50 ^{bc}	71.20 ^{ab}	66.87 ^{bc}	62.06 ^{cd}	56.90 ^d	7.12	0.0001	0.952	0.263
ADFI (kg/DM)	1.75 ^{ab}	1.77 ^{ab}	1.75 ^{ab}	1.85 ^a	1.87 ^a	1.59 ^c	1.49 ^c	0.18	<.0001	0.0047	<.0001
ADG(kg)	0.85 ^a	0.81 ^{ab}	0.83 ^{ab}	0.83 ^{ab}	0.81 ^{ab}	0.72 ^c	0.71 ^c	0.09	<.0001	0.898	0.192
FCR	2.06 ^d	2.16 ^b	2.13 ^{bc}	2.39 ^a	2.25 ^a	2.11 ^c	2.07 ^d	0.21	<.0001	0.045	<.0001

^{a,b} Within a row means with different superscripts differ ($P < 0.001$), ¹CON – Control diet; LFC – Liquid fermented control diet, LFCE- Liquid fermented control with enzyme diet, LLFPHE- Low liquid fermented potato hash with enzyme diet, LLFPH- Low liquid fermented potato hash without enzyme diet, HLFPH- High liquid fermented potato hash with enzyme diet, HLFPH- High liquid fermented potato hash without enzyme diet. ²IW- Initial weight, FW – Final weight; ADFI- Average daily feed intake, ADG- Average daily gain, FCR – Feed gain ratio

5.4 Results

The weekly as fed feed intake (WFI) of grower pigs fed diets based on incremental inclusion level of liquid fermented potato hash treated with or without enzyme during the 8-week period are illustrated in Figure 5.1. As was expected, control had a higher ($P < 0.001$) feed intake during week 1 as compared to liquid fermented diets. However, during week 1, LFPH+E had higher ($P < 0.001$) feed intake than LFC, LFC+E, LLFPH, HLFPH+E and HLFPH. During week 2, control, LFC, LLFPH+E, LLFPH consumed ($P < 0.001$) more feed than LFC+E, HLFPH+E and HLFPH. However, from week 3 to week 8 control, LFC, LLFPH+E, LLFPH, HLFPH+E and HLFPH had higher ($P < 0.001$) WFI compared to LFC+E. For all the treatments, WFI was reduced from week 6 to week 8.

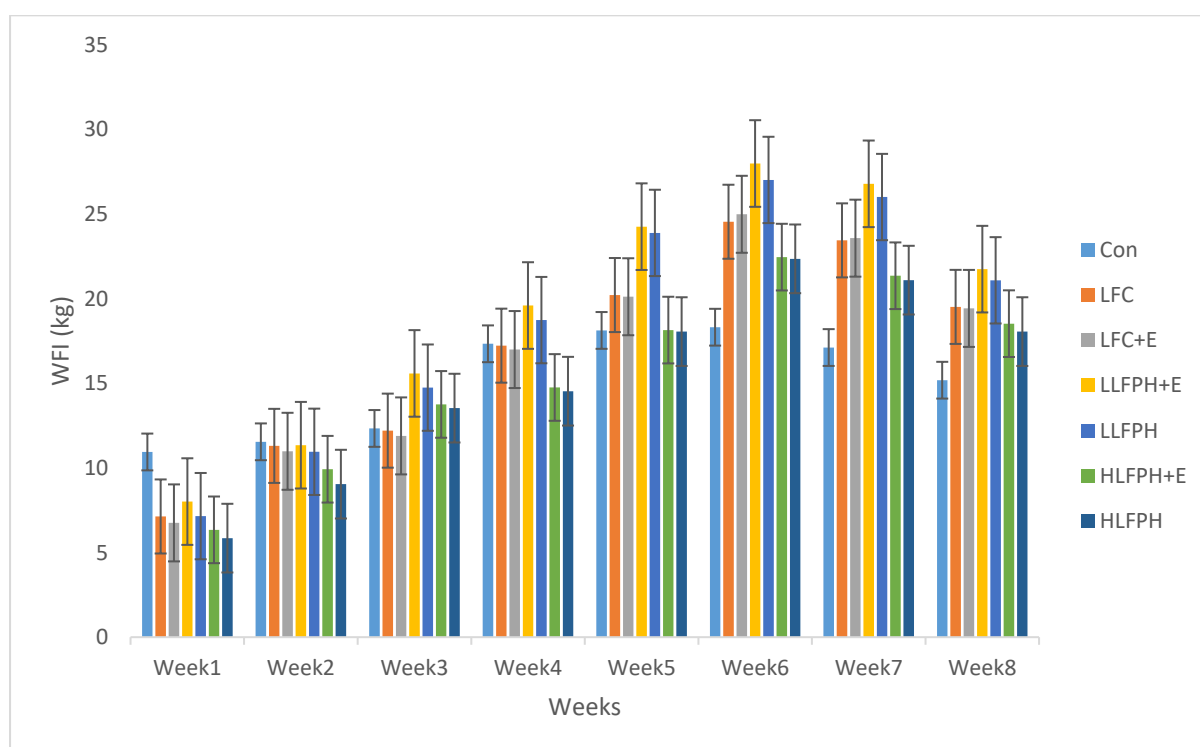


Figure 5.1: Weekly changes in feed intake of pigs fed diets containing different inclusion of liquid fermented potato hash with or without enzyme.

{Con- control; LFC- Liquid fermented control; LFC+E- Liquid fermented control with enzyme; LLFPH+E- Low liquid fermented potato hash with enzyme; LLFPH- Low liquid fermented potato hash without enzyme; HLFPH+E- High liquid fermented potato hash with enzyme; HLFPH- High liquid fermented potato hash without enzyme}

Growth performance of LW×LR crossbred pigs fed control, FLC, LFLPH and HFLPH treated with or without enzyme diets are shown in Table 5.1. There was no difference ($P > 0.05$) in initial body weight between the treatments. However, control and LLFPH+E had higher ($P < 0.05$) final weight compared to LFC, LFC+E, LLFPH, HLFPH+E and HLFPH treatments. Pigs that were fed control, LFC, LFC+E, LFPH consumed more feed ($P < 0.05$) than pigs that were fed HLFPH+E and HLFPH. However, pigs that were fed LLFPH+E and LLFPH had a better feed conversion ratio compared to pigs that were fed control, LFC, LFC+E, HLFPH+E and HLFPH. There were no differences ($P > 0.05$) in diet × enzyme interactions for IW, FW and ADG. However, there was a difference ($P < 0.05$) in diet × enzyme interactions and pigs fed fermented liquid diets treated with enzyme had lower ADFI and higher FCR than pigs fed liquid fermented diets without enzyme.

5.5 Discussion

Feeding fermented liquid feed to pigs has been proposed as an approach to maintain high and regular feed and water intake. The fermentation of a liquid diet has been reported to influence the physiology, microbiology and morphology of the GIT (van Winsen *et al.*, 2001; Scholten *et al.*, 2002; Canibe and Jensen, 2003). It has also been shown to decrease the incidence of diarrhoea (Højberg *et al.*, 2003) and pig dysentery (Lindecrona *et al.*, 2003), as well as the spread of zoonoses to the consumers (Brooks *et al.*, 2003), as compared with dry feed. In the current study, pigs did not have any problem with diarrhoea or any health related problems which is consistent with those previous studies.

Results of the current study showed that the ADG, ADFI, and FCR were not affected by inclusion of low fermented liquid potato hash diets treated with or without enzyme.

Feeding a liquid diet to pigs has been generally reported to improve performance compared with feeding a dry diet (Russell *et al.*, 1996; Kim *et al.*, 2001; Choct *et al.*, 2004a; Han *et al.*, 2006), although the studies of Lawlor *et al.* (2002) and Pedersen *et al.* (2005) did not find any particular benefit in feeding fermented liquid feed. The process of fermentation converts the starch and sugars of potato hash into volatile fatty acids, alcohol and lactic acid (Prescott *et al.*, 1996). These fermentation products are considered highly palatable and may have the potential to increase the feed intake of the pigs (Scholten *et al.*, 1999). Growing pigs reared under commercial conditions can record minimum daily gains of 630 g per day⁻¹ when fed on concentrate diets (Hoffman *et al.*, 2003). However, the present study recorded daily gains of > 720 g day⁻¹ which is higher than those reported in our previous study (Thomas *et al.*, 2011) when 200 and 400 g kg⁻¹ potato hash silage was included in the diet of pigs. In addition, Nkosi *et al.* (2010) recorded lower daily gains <120 g day⁻¹ when ensiled total mixed potato hash ration with or without bacterial inoculation was fed to growing crossbred (Large White x Landrace) pigs.

The current study showed that low fermented liquid potato hash feed improved growth performance of grower pigs. In addition, in the current study low fermented liquid potato hash showed an improved FCR compared to dry feed. This agrees with the improved G: F measured by Scholten (2001) who fed liquid feed containing fermented wheat compared to feeding non-fermented liquid feed. In addition, Pedersen (2006) measured higher G: F in pigs fed liquid feed containing fermented liquid grain compared to non- fermented liquid feed. Scholten *et al.* (2002) reported that the G:F was improved when pigs were fed liquid feed containing 45 % fermented wheat

compared with those fed non fermented liquid feed. Pedersen (2006) also reported that pigs fed diets containing 66 % fermented liquid grain improved the G: F of pigs.

In the current study, the DMI was improved in pigs fed the low fermented liquid potato hash diet, which may be the reason for an improved ADG compared with pigs fed the control diet. Brooks *et al.* (2001) reported that fermented cereal grains could decrease the pH value, and improve the palatability of fermented liquid feed. Boesen *et al.* (2004) also reported that fermented liquid diets decreased gastric pH and increased gastric lactic acid concentration in pigs. In this study the low fermented liquid potato hash diet had a lower pH Value. We hypothesized that this may be the other reason that ADG was improved in pigs fed the low fermented liquid potato hash diet. The reduction in FI, ADG, FW and FCR in the high inclusion level of fermented liquid potato hash treated with or without enzyme indicates that replacing non-fibrous ingredients with bulky feedstuffs limits intake (Nyachoti *et al.*, 2004). The high inclusion level of liquid fermented potato hash treated with or without enzyme had a higher NDF and the feed was also bulker. The bulkiness of the feeds is a result of an increase in fibre inclusion level, which enhances the ability of the feed, especially non-starch polysaccharides, to trap water within its matrices, swell and form gels with high water contents (Robertson and Eastwood, 1981).

The level of NSPs in the diets used in the current study were, however, not determined. During transit of digesta in the GIT, feed particles tend to absorb water within their matrices and swell, thereby filling the gut quickly and reducing space for more feed to be consumed (Whittemore *et al.*, 2003b; Anguita *et al.*, 2006). The reduction in FI as WHC increased suggests that the gut of finishing pigs developed mechanisms to cope

with fibre-rich diets, thereby altering the WHC of the feed. Considering that CP was low in these diets with high liquid fermented potato hash diets, the effect of CP was likely to have been confounded with NDF and CF contents of these feeds. Evidence exists that an average of 31 % of protein in commonly used fibre sources is bound to the NDF and is not available for the pig (Bindelle *et al.*, 2005). Besides, dietary fibre inclusion in the feed reduces protein concentration and may disturb the balance between amino acid concentrations. Incorporation of high inclusion level of potato hash in the current study could have physiologically contributed in depressing intake by disturbing the balance in nutrients. Henry *et al.* (1992) observed that low dietary ratio of tryptophan to large neutral amino acids reduce feed intake.

As the dietary fibre increased, FI was initially reduced and then reached an equilibrium point where intake became constant. At that point, the coefficient describing the rate of change of FI with the increase in bulkiness was closer to zero for CF than for NDF, suggesting that this physicochemical measurement of bulk could have been assisted by another dietary property such as water intake in constraining gut capacity. Water consumption was, however, not captured in the current study. Allen (2000) also reported negative relationships between dietary NDF concentration and intake. The NDF content is a major factor determining gut fill and can be used to predict the effect the bulkiness of a feed has on the gut capacity. Increase in NDF and CF concentrations in feeds impose some masking effects on the availability of feed ingredients for enzymatic degradation and increase the transit time in the small and large intestines (Le Goff *et al.*, 2003). A diet with low CF or higher NDF concentration occupies lesser space than a diet with high CF or low NDF (Le Goff *et al.*, 2003).

Hence, gut capacity is easily attained for high CF and NDF thereby reducing feed intake.

Adding the enzyme to liquid fermented potato hash diet improved feed intake and feed conversion ratio over 13 % in the current experiment. This result is consistent with previous reports (Pettey *et al.*, 2002; Fang *et al.*, 2007; Ji *et al.*, 2008). Kim *et al.* (2003) reported that supplementing carbohydrase in corn-soybean based-diets to nursery pigs improved gain: feed ratio by 9 %. The greater improvement in FCR obtained in this study may be due to the different enzyme used. In this study, enzyme contained xylanase, amylase and protease which targeted substrates including NSP, indigestible starch and protein. While in the experiment of Kim *et al.* (2003), the enzyme used contained β -1,4-mannanase, β -1,4-mannosidase and β -1,6-galactosidase, which only targeted NSPs. Enzyme supplementation improved the digestibility of gross energy, crude protein, and dry matter. This result is consistent with previous findings (Caine *et al.*, 1998; Café *et al.*, 2002; Olukosi *et al.*, 2007).

5.6 Conclusions

It was concluded that the supplementation of enzyme on liquid fermented diets improved growth performance of pigs. The performance of pigs fed low inclusion level of fermented liquid potato hash diets treated with or without enzyme did not negatively impact growth performance of grower pigs. Therefore, since the inclusion level of liquid fermented potato hash diets treated with or without enzyme did not affect negatively the growth performance of grower pigs, this implies that different inclusion level of liquid fermented potato hash are essential as pig feed.

5.7 References

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Chapter 6

Effect of different inclusion levels of fermented potato hash with or without exogenous enzymes on carcass characteristics of Large White × Land-race crossbred pigs

Abstract

An experiment was conducted to assess carcass traits of cross-breed (Landrace X Large White) pigs fed varying levels of liquid potato hash based diets fermented with or without enzyme. Forty-two grower pigs, aged 130 days at an average live weight of 60 ± 4 kg were fed on diets containing CON (control diet that was not fermented and without potato hash), fermented Liquid control diet (LFC), LFPH (diet containing 200 g potato hash.kg⁻¹ diet, as fed), HFPH (diet containing 400 g potato hash.kg⁻¹ diet, as fed), fermented control diet treated with an exogenous xylanase enzyme (Natugrain TS L®), LFPHE (diet containing 200 g potato hash.kg⁻¹ diet treated with an exogenous xylanase enzyme (Natugrain TS L®), as fed), HFPHE (diet containing 400 g potato hash.kg⁻¹ diet treated with an exogenous xylanase enzyme (Natugrain TS L®), as fed) were slaughtered and carcass traits were evaluated. Pigs that were fed the control diet had higher ($P < 0.05$) slaughter weight than those that were fed on diets containing fermented liquid feed. There were no differences ($P > 0.05$) in warm, cold carcass weights dressing percentage and carcass length between diets containing different inclusion levels of fermented liquid potato with or without enzyme and control. Low fermented liquid potato hash diet with or without enzyme had a higher ($P < 0.05$) drip loss percentage, eye muscle area and lower back-fat thickness and shoulder fat measurements than control, liquid fermented control and high liquid fermented potato hash with or without enzyme. Exogenous enzymes had a positive impact on pork cuts. However, low fermented liquid potato hash diets with or without enzyme had a higher

($P < 0.05$) rib mass portion and hind quarter portion compared to other treatments. Dietary inclusion of low fermented liquid potato hash treated with or without enzyme improved carcass traits of growing pigs. Further work that will evaluate the effects of fermented liquid potato hash with or without enzyme on meat quality of baconers is warranted since the age of a pigs plays a major role in meat quality.

Keywords: Potato hash, grower pigs, liquid fermentation, enzyme carcass traits

6.1 Introduction

The conventional way of feeding pigs based on soybean meal, corn or sorghum grain, which are becoming expensive; therefore, the pig industry is including by-products from food processing as a strategy to maintain their production costs. By-products from the food processing industry are high in moisture (Kayouli and Lee, 1999). Moist feeds decay readily, producing nutrient losses and contamination with microorganisms and their toxins in the process. Controlled fermentation is an attractive option for preserving these high moisture by-products. Fermented liquid diets (FLD) are a tool lowering stomach pH, through increased lactic acid concentration and help to decrease populations of pathogens such as *E. coli* and *Salmonella* spp. (Lindecrona *et al.*, 2000; Mikkelsen and Jensen, 2000). Potato hash, a mixture of potato skins, starch, fats and yellow maize obtained after the production of snacks, is one of the agro-industrial by-products that is available in appreciable quantities in South Africa. This by-product contains 150 g DM/kg of fresh potato hash, 700 g starch/kg DM, 11.16 MJ ME/kg DM, 105 g CP/kg DM, 369.6 g NDF/kg DM and 162.5 g ADF/kg DM. It has been reported that fermenting high moisture by-products can replace costly feeds such as maize in the diets (Itavo *et al.*, 2000, Lallo *et al.*, 2003, Pirmohammadi *et al.*, 2006).

Performance indicators such as body weight gain, feed conversion ratio, feed efficiency and feed cost /kilogramme weight gain have often measured the potential use of these unconventional feed ingredients. Of much importance to the health and sensory appeal of the consumers of animal products on one hand and the producers of the products on the other hand are such carcass indices like fat content, protein content, carcass yield and the lean to fat ratio of the carcass of animals fed with these unconventional feed ingredients. Sarker *et al.* (2010) reported fed unconventional feed

ingredients from to pigs and reported thinner back fat, higher protein and moisture and lower crude fat in their carcasses. The effect of the use of fermented sweet orange peel on carcass quality of broilers was investigated by Oluremi *et al.* (2010). These authors reported a significant effect on the birds dressing percentage and concluded that 30 % replacement of maize with naturally fermented sweet orange peel meal is practicable in broiler production enterprise. The dietary use of fermented liquid potato hash treated with or with enzyme fed grower pigs has not been investigated. Thus, the objective of the study was to compare carcass characteristics of pigs fed liquid fermented potato hash.

6.2 Materials and methods

6.2.1 Pigs, treatments and experimental design

Forty-two (six pigs per treatment) crossbred male pigs (Large White x Landrace) aged 55 days with average weight of 25.5 ± 3 kg that were used for growth performance trial (Chapter 5) were used for this study. These pigs were humanely slaughtered when they attained a weight of 60 ± 4 kg. The seven diets as described in chapter 5 were fed to grower pigs. Fermented liquid diets were prepared by mixing diet with water, at a ratio of 1:2. The effect of these diets on carcass characteristics in growing pigs were evaluated. The experimental procedures described in the study were approved by the Animal Ethics Committee of the Agricultural Research Council, Animal Production Institute (ARC-API).

6.2.2 Measurements

6.2.2.1 Pre-slaughter activities

Pigs were weighed prior to slaughtering, transported simultaneously in the early hours (7:30 am) of the morning to the ARC–Irene abattoir where they were provided with fresh drinking water, kept calm and then slaughtered. At the abattoir, standard pre-slaughtering procedures were followed.

6.2.2.2 Slaughtering

Each pig was electrically stunned with an electrical stunner set at 220 V and 1.8 A with a current flow for 6 seconds. Electrical stunner electrodes were positioned at the base of each ear. Exsanguination followed within 10 seconds after stunning. Dehairing and evisceration were done according to the abattoir's standard operating procedures.

6.2.2.3 Post-slaughter activities

After slaughtering, standard abattoir post-slaughtering procedures were followed. Warm carcass weight (WCW) was measured after dressing using an overhead scale. Dressing percentage (DP) was determined as the warm carcass weight as a percentage of live weight. Carcasses were then placed in a cold room, kept at an approximate temperature of 0 °C for 24 hours after which cold carcass weights (CCW) were measured. The head from each carcass was then removed at the atlanto-occipital joint, the tail at the junction of the third and fourth sacral vertebrae and the flare fat, kidneys, kidney fat, glands and remaining parts of the diaphragm were also removed. Carcasses were then split into two parts along the median plane from the remaining sacral vertebra to the first cervical vertebra with a carcass splitting band

saw. Carcass length (CL) was measured from the first rib to the pubic bone using a measuring tape. Backfat measurements were taken at first rib (DFT1), last rib (DFT2) and last lumbar vertebra (DFT3). All other carcass measurements were taken from the left side. A cut was made between the 10th and 11th ribs and carried on through the spinal column. The P2 fat measurement was taken on each carcass with vernier callipers over the eye muscle, 60 mm from the carcass midline. Eye muscle length (EML) and three measurements of the eye muscle width (EMW) were taken from the cut interface. Lean meat percentage (Lean %) was calculated using the formula of Bruwer (1992) presented below:

$$\text{Lean \%} = 72.5114 - 0.4618V + 0.0547S$$

where V is the fat thickness (mm) and S is the muscle depth (mm).

The eye muscle area (EMA) was estimated using the formula proposed by Zhang *et al.* (2007) as:

$$\text{EMA} = \text{EML} \times \text{EMW} \times 0.7$$

Where EMW was the average of the three width measurements of the eye muscle

From the same cut where P2 measurements were taken, a sample joint measuring 2.5 cm thick and 16 cm long measured along the surface of the back of the eye muscle was cut out and weighed. This sample joint was placed in a netlon bag and inserted in a small plastic bag which was then tied in such a way as to prevent the sample joint from touching the bottom of the plastic bag or air coming into the bag. They were then stored in a refrigerator between 0 and 5 °C for 24 hr after which the mass of the water

lost was calculated from the weight of the water in the bag and used to calculate drip loss (DL).

Thereafter, the primal cuts (shoulder, hindquarter and rib) from the carcasses were removed on a stationary band saw. The shoulder was removed by cutting between the third and fourth ribs caudally and the junction of the caudal edge of the second rib with the sternum cranially, with the front trotter removed by cutting through the metacarpal region (at the joint of the carpal bones and the radius and ulna) and weighed to get shoulder weight (SW). The rib was cut from between the fourth and twelfth thoracic vertebrae dorsally and along a parallel line 16 cm from the spinal cord midline ventrally. It was weighed to obtain the rib weight (RW).

The hind leg was removed between the second and third sacral vertebrae perpendicular to the stretched leg and at the hock joint distally and weighed to get the hindquarter weight (HQW). It was also measured to get the hindquarter length (HQL), from the ischiopubic symphysis to the hock joint and the hindquarter circumference (HQC) in the area of maximum amplitude near the base of the tail. The RW, SW and HQW were then each presented as a proportion of CCW to give RWP, SWP and HQWP, respectively.

6.2.2.4 Statistical analyses

Data for carcass traits of pigs fed on fermented liquid potato hash diets treated with or enzyme was analysed for effects of treatment using General Linear Model (GLM) procedures of SAS (SAS Inst. Inc., Cary, NC). All data were tested for normality and

homogeneity and comparisons were made to the 95 % significance level. The model used was:

$$Y_{ijkl} = \mu + \alpha_i + \beta_j + (\alpha \times \beta)_{ij} + \epsilon_{ijk};$$

where:

Y_{ijkl} is the carcass traits;

μ is the overall mean response common to all observations;

α_i is the effect of inclusion level;

β_j is the effect of enzyme ($j = E$);

$(\alpha \times \beta)_{ij}$ is the interaction between the enzyme and inclusion level and ϵ_{ijk} is the residual error.

6.4 Results

Carcass traits of LW x LR crossbred pigs fed control, FLC, LFLPH and HFLPH treated with or without enzyme diets are shown in Table 6.1. There was no difference ($P < 0.05$) between treatments on WCW, CCW, DP% and CL. However, HLFPH+E, HLFPH and LFPH diets had higher ($P > 0.05$) DL% compared to control, LFC, LFC+E and LLFPH+E. In addition, HLFPH+E, HLFPH, LLFPH+E, LFPH and LFC+E diets had a greater ($P > 0.05$) lean % compared to control. However, carcasses that were fed HLFPH+E, HLFPH, LLFPH+E, LFPH and LFC+E had lower ($P > 0.05$) back-fat (BF), P2 fat and shoulder fat compared to control and LFC. Carcasses that were fed LLFPH+E, LLFPH, LFC and LFC+E had greater ($P > 0.05$) EMA than carcasses that were fed HLFPH+E, HLFPH and control. There was no difference ($P < 0.05$) in CL among treatments. There was no diet x enzyme ($P < 0.05$) interactions for WCW, CCW, DL%, DP%, Lean%, EMA, BF, shoulder fat and CL.

Primal pork cuts measurements of grower LW x LR crossbred pigs fed control, FLC, LFLPH and HFLPH treated with or without enzyme diets are shown in Table 6.2. There was no difference ($P < 0.05$) between carcasses that were fed diets containing control, FLC, LFLPH and HFLPH treated with or without enzyme on shoulder mass and hind quarter mass. However, carcasses that were fed LLFPH+E, LLFPH, LFC+E and HLFPH had a higher ($P > 0.05$) rib mass portion compared to carcasses that were fed control and LFC. In addition, carcasses on control, LFC, LFC+E, LLFPH+E and LLFPH had greater ($P > 0.05$) ham diameter than carcasses on HLFPH+E and HLFPH. There was no diet x enzyme ($P < 0.05$) interactions for WCW, CCW, DL%, DP%, Lean%, EMA, BF, shoulder fat and CL.

Table 6.1: Carcass traits of Large White x Landrace (LW x LR) pigs fed diets containing different inclusion levels of liquid fermented potato hash with or without enzyme

Parameters ²	Treatments ¹								P		
	CON	LFC	LFC+E	LLFPH+E	LLFPH	HLFPH+E	HLFPH	SEM	Diet	Enzyme	Diet*Enzyme
WCW, kg	48.65	46.60	42.60	50.95	44.18	44.73	43.15	7.19	0.485	0.732	0.226
CCW, kg	42.50	45.41	43.60	49.53	42.87	43.60	41.90	8.14	0.718	0.779	0.316
DL%	2.41 ^c	2.39 ^c	2.43 ^c	2.71 ^{ab}	2.75 ^{ab}	2.91 ^a	3.04 ^a	0.42	0.0003	0.395	0.129
DP%	69.95	69.89	69.98	71.01	71.04	70.98	70.97	9.16	0.453	0.614	0.792
Lean%	60.98 ^b	61.01 ^{ab}	61.91 ^a	64.98 ^{ab}	65.15 ^{ab}	67.84 ^a	68.01 ^a	1.75	0.0001	0.349	0.661
EMA, cm ²	9.99 ^{bc}	10.65 ^{ba}	10.55 ^{ba}	11.76 ^a	10.77 ^{ba}	9.32 ^c	9.30 ^c	5.64	<.0001	0.044	0.085
BF, mm	8.52 ^a	8.44 ^a	7.32 ^b	7.04 ^b	7.09 ^b	6.91 ^b	6.941 ^b	3.18	0.023	0.071	0.019
P2, mm	7.91 ^a	7.46 ^a	5.56 ^c	5.04 ^b	5.09 ^b	4.71 ^b	4.92 ^b	3.37	<.0001	0.385	0.782
Should fat, mm	18.83 ^a	18.83 ^a	15.98 ^b	15.16 ^b	15.78 ^b	15.00 ^b	15.16 ^b	6.15	<.0001	0.79	0.395
CL, cm	82.33	81.35	78.00	79.67	84.33	80.50	82.36	4.68	0.575	0.916	0.098

^{a,b} Means with different letters in a row differ significantly ($P < 0.0001$); ¹CON – Control diet; LFC- Liquid fermented control diet; LFC+E- Liquid fermented control with enzyme diet; LLFPH- Low liquid fermented potato hash with enzyme diet; LLFPH- Low liquid fermented potato hash without diet; HLFPH-High liquid fermented potato hash with enzyme diet; HLFPH- High liquid fermented potato hash without diet; ²WCW- warm carcass weights; CCW- cold carcass weights; DP - dressing percentage; CL - carcass length; P2 – back-fat thickness; EMA - eye muscle area; Lean % - lean percentage; DL - drip loss

Table 6.2: Primal pork cuts measurements of Large White x Landrace (LW x LR) pigs fed diets containing liquid fermented potato hash at low (LLFPH) and high (HLFPH) inclusion levels with or without enzyme

Parameters ¹	Treatments								P		
	CON	LFC	LFC+E	LLFPH+E	LLFPH	HLFPH+E	HLFPH	SEM	Diet	Enzyme	Diet*Enzyme
SWP%	14.16	14.19	3.98	14.00	14.21	14.25	14.19	1.27	0.776	0.881	0.123
RWP%	11.51 ^c	11.01 ^c	12.59 ^{ab}	13.43 ^a	13.41 ^a	12.65 ^{ab}	12.00 ^b	0.69	0.0001	0.337	0.643
HQL, cm	10.55	10.41	10.52	10.62	10.49	10.38	9.75	2.13	0.465	0.661	0.882
HC, cm	59.83 ^{ab}	58.96 ^b	59.64 ^{ab}	61.00 ^a	60.98 ^a	57.20 ^c	57.16 ^c	4.07	<.0001	0.335	0.881
HL, cm	31.06	32.00	32.00	32.13	31.98	32.23	32.00	2.05	0.765	0.346	0.158

^{a,b} Means with different letters in a column differ significantly ($P < 0.0001$); ¹HQL - hind quarter length; HC - ham circumference; HL – ham length; RWP - rib weight proportion; SWP- shoulder weight proportion; ²CON – Control diet; LFC- Liquid fermented control diet; LFCE- Liquid fermented control with enzyme diet; LLFPH- Low liquid fermented potato hash with enzyme diet; LLFPH- Low liquid fermented potato hash without diet; HLFPH-High liquid fermented potato hash with enzyme diet; HLFPH- High liquid fermented potato hash without diet

6.5 Discussion

The warm carcass and cold carcass weights were not significantly different among treatments in this study. Similar results were found in studies where pigs were fed high-fibre diets (Jorgensen *et al.*, 1996). Pelevina (2007) also reported similar responses when pigs were fed with dry brewer's grain. Altizio *et al.* (2000) reported that pigs fed diets with 25 % wet brewer's grain tended to produce leaner carcasses, even though carcass dressing and primary cuts were similar. Urlings *et al.* (1993) reported that feeding fermented feed increased back-fat thickness and decreased meat percentage in finishing pigs. In the present study, different inclusion levels of fermented liquid potato hash with or without enzymes reduced back-fat measurements.

The low fermented liquid potato hash with or without enzyme diets had greater carcass weights than high fermented liquid potato hash with or without enzyme diets. This could be interpreted as the high liquid fermented potato hash with or without enzyme diets having high fibre. Alternatively, it could be that diets with higher fermented liquid potato hash with or without enzymes did not supply sufficient nutrients to meet the requirements of these pigs. High fibre diets increase weights of visceral organs in pigs (Montagne *et al.*, 2003).

Diets containing different inclusion levels of fermented liquid potato hash with or without enzyme did not have a lower DP as was expected. This is in agreement with the previous study (Thomas *et al.*, 2013) where there was no differences in DP of pigs fed different inclusion levels of potato hash silage and a study by Borton and Rahnema (1998), where there was no differences in DP of pigs fed potato chip scraps. In

addition, Scipioni and Martelli (2001) found no differences in carcass traits of pigs fed 10% and 20% sugar beet pulp diets. Hang (1998), reported that the inclusion of 5% ensiled cassava leaves in the diet of growing pigs did not affect carcass traits. Kim *et al.* (2006), who experimented by feeding the Berkshire breed of pigs with fermented persimmon shell diet, reports that the fermented persimmon shell diet reduced the moisture content and increased the crude fat of pork. In the results of the present study, the intake of different inclusion levels of fermented liquid potato hash with or without enzyme was found to affect the chemical composition of carcass traits, which is similar to the report of Kim *et al.* (2006).

Pigs fed low and high fermented liquid potato hash with or without enzyme had greater drip loss values than pigs fed control, fermented liquid control with or without enzyme. Drip loss (DL) of pork fed high moisture diets is affected by numerous and complex factors including rate of pH decline and ultimate pH, presence of the halothane gene and transportation among others (Pérez *et al.*, 2002; Rosenvold and Andersen, 2003; Fischer, 2007). Although drip loss is of economic importance, the mechanism behind this phenomenon has not been adequately studied (Otto *et al.*, 2007). In addition, because the high fermented liquid potato hash with or without enzyme had a greater ultimate pH, the DL results contradict the assertion by Huff-Longergan *et al.* (2002) that a higher ultimate pH is associated with better water holding capacity, translating into lower drip losses during storage.

Our diets did not influenced length of the carcass in the study. Carcass length affected the weights of the most valuable meat cuts (Poto *et al.*, 2007) and determined the

amount of rashers of back bacon obtained (Kanengoni *et al.*, 2004). English *et al.* (1988) stated that carcass weight and the genotype of the pig largely influence CL.

The control, fermented liquid control with or without enzyme had more subcutaneous fat than the low and high liquid fermented potato hash with or without enzyme and this showed in a lower lean percentage. Back-fat thickness, P2 and shoulder fat measures were lower in fermented liquid potato hash with or without enzyme compared to control, and fermented liquid control diets. Inclusion levels of liquid fermented potato hash with or without enzyme in diets however did not negatively affect some measurements of selected important commercial pork cuts in South Africa comprising the hindquarter, ham length and shoulder. The hindquarter length, circumference and weight proportion and the shoulder weight proportion measures were affected mainly by diets.

6.6 Conclusions

This study revealed potential benefits that could be harnessed through the use of fermented liquid potato hash in terms of meeting the demand for carcass leanness. Dietary inclusion of low fermented liquid potato hash treated with or without enzyme improved carcass traits of growing pigs. Further work that will evaluate the effects of liquid fermented potato hash with or without enzyme on meat quality of baconers is warranted since the age of the pigs plays a major role in meat quality.

6.7 References

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CHAPTER 7

General Discussion, Conclusions and Recommendations

7.1 General discussion

Competition with humans and the biofuel industry for feed resources is constraining the pig industry, which already has a narrow range of feed ingredients (Smale *et al.*, 2013). Potato hash a by-product from Simba is an underutilized feed resource that can be incorporated into pig feed. This study proposed that potato hash be regarded as a valuable pig feed ingredient and innovative ways be applied to increase its utilisation. It was proposed that potato hash should be processed through fermentation with exogenous enzymes to reduce the fibre levels before embarking on feeding trials.

The hypothesis tested in Chapter 3 was that physicochemical properties and nutrient composition of varying levels of liquid fermented potato hash diet are highly variable. Seven dietary treatments were formulated and treatments were: CON (control diet that is not fermented and without potato hash), LFC (Liquid fermented control diet), LLPH (diet containing 200 g potato hash.kg⁻¹ diet, as fed), HLFPH (diet containing 400 g potato hash.kg⁻¹ diet, as fed), LFCE (fermented control diet treated with an exogenous xylanase enzyme (Natugrain TS L®), LLFPHE (diet containing 200 g potato hash.kg⁻¹ diet treated with the exogenous xylanase enzyme, HLFPE (diet containing 400 g potato hash.kg⁻¹ diet treated with an exogenous xylanase enzyme. The LLFPH+E and HLFPH+E diets had a higher ($P < 0.05$) DM, CP, WHC and lower ($P < 0.05$) NDF, ADF and SC compared to LLFPH and HLFPH diets. However, there were no difference ($P < 0.05$) on EE, GE and density between the diets.

Chapter 4 tested the hypothesis that liquid fermented potato hash diets have variable microbial characteristics. Six treatments, LFC (Liquid fermented control diet), LLPH (diet containing 200 g potato hash.kg⁻¹ diet, as fed), HLFPH (diet containing 400 g potato hash.kg⁻¹ diet, as fed), LFCE (fermented control diet treated with an exogenous xylanase enzyme (Natugrain TS L®), LLFPHE (diet containing 200 g potato hash.kg⁻¹ diet treated with the exogenous xylanase enzyme), HLFPE (diet containing 400 g potato hash.kg⁻¹ diet treated with an exogenous xylanase enzyme) were assessed. The liquid fermented control with enzyme (LFC+E), low liquid fermented potato hash with enzyme (LLFPH+E) and high liquid fermented potato hash with enzyme (HLFPH+E) had lower pH values at 8 and 24 hrs ($P < 0.05$) compared to liquid fermented control (LFC), low liquid fermented potato hash (LLFPH) and high liquid fermented potato hash (HLFPH). Enzyme treated liquid fermented potato hash diets reduced pH and increased WSC rapidly than liquid fermented potato hash without enzyme. The number of lactic acid bacteria in the LFC+E, LLFPH+E and HLFPH+E was increased compared to LFC, LLFPH and HLFPH. Enterobacteriaceae counts bacteria in the LFC+E, LLFPH+E and HLFPH+E was decreased compared to LFC, LLFPH and HLFPH.

The Chapter 5 tested the hypothesis that Liquid fermented feed improves feed intake and utilization of feed by grower pigs. Forty-two (six pigs per treatment) crossbred male pigs (Large White x Landrace) aged 55 days with average weight of 25.5 ± 3 kg were fed diets containing varying levels of fermented liquid potato hash. The LLFPH+E had greater ($P < 0.05$) final weight (FW), average daily feed intake (ADFI), dry matter intake (DMI), average daily gain (ADG) and feed conversion ratio (FCR) than the LLFPH, HLFPE, LFC. There was no difference ($P < 0.05$) between LLFPHE and

control diets. The fermented liquid diets treated with enzyme improved ($P < 0.05$) feed intake of grower pigs. There were diet x enzyme interactions for average daily intake and feed conversion ratio in the growing pigs.

Chapter 6 tested the hypothesis that inclusion levels of liquid fermented potato hash feed influence carcass characteristics of grower pigs. Carcass characteristics of grower pigs fed varying levels of fermented liquid potato hash with or without enzymes were assessed. Pigs that were fed the control diet had higher ($P < 0.05$) slaughter weight than those that were fed on diets containing fermented liquid feed. There were no differences ($P > 0.05$) in warm, cold carcass weights dressing percentage and carcass length between diets containing different inclusion levels of fermented liquid potato with or without enzyme and control. Low fermented liquid potato hash diet with or without enzyme had a higher ($P < 0.05$) drip loss percentage, eye muscle area and lower back-fat thickness and shoulder fat measurements than control, liquid fermented control and high liquid fermented potato hash with or without enzyme. Exogenous enzymes had a positive impact on pork cuts. However, low fermented liquid potato hash diets with or without enzyme had a higher ($P < 0.05$) rib mass portion and hind quarter portion compared to other treatments.

Based on the findings of the present study, it is feasible to produce liquid feed using potato hash as a pig feed ingredient. This is because no health problems occurred when feeding fermented liquid PH to grower pigs, and pig performance was comparable to that of control diet. It should however, be noted that the production of fermented liquid feed requires good management skills since poor fermentation may lead to poor feed quality, which will adversely affect the pigs to be fed. Pig farmers who are currently feeding PH to pigs, must be taught on the technology of liquid

fermentation from PH. This will capacitate them to produce fermented liquid feed independently at their farms. Furthermore, the use of PH in the form of liquid fermented diet in pig nutrition will reduce environmental pollution that may occur due to dumping.

7.2 Recommendations and further research

One of the major drawbacks for the use of potato hash in pig nutrition is its low DM (150 g DM/kg) and CP (105 g CP/kg DM) contents. However, the present study showed that this can be corrected by feeding potato hash as a liquid diet to supply enough nutrients that are required for optimal pig production. Due to the fact that the production of meal from high moisture by-products needs machinery facilities and may not be affordable to farmers, fermentation of potato hash was chosen as the cheapest method that can be adopted by the farmers. The present study showed that potato hash can be fermented successfully with the addition of an exogenous enzyme.

Diets containing potato hash were formulated with or without enzyme and mixed with water at a ratio of 1:2 and fermented in 100 L drums. A back-slope method was used to produce liquid fermented potato hash diets. Diets were fermented for 8 hrs before being fed to pigs. The study showed that the addition of enzyme during the fermentation of potato hash diets reduced the pH, increase lactic acid concentration and water soluble carbohydrates. However, further work is needed in which identification of the microbial populations in fermented liquid potato hash to species level and quantification of metabolites from carbohydrate and amino acid fermentation. With this knowledge, strategies that promote beneficial species and that impede the proliferation of undesired species resulting in FLF with optimal microbial characteristics will be much easier to establish.

It is generally known that maize, wheat and sorghum are typically used in pig feed. This practice is generally not attainable for the smallholder farmers. Some reasons being that i) some farmers who are residing in proximity with the high industrialized areas or urban centres may lack with available land for crop production and ii) forages such as maize are primarily produced for human consumption and the production of silage from maize may be an expensive commodity. As a result, the present study compared varying levels of fermented liquid potato hash with or without enzyme on the growth performance of Large White × Landrace crossbred pigs. The results of the present study showed that fermented liquid potato hash that was treated with or without enzyme at 20 % inclusion levels can be included in the diet of growing pigs. However, further work is needed for further research to characterize physicochemical properties of digesta in different sections of the gut of pigs fed fermented liquid potato hash. In addition, there is a need for further research on nutrients digestibility and rate of passage of growing pigs fed on fermented liquid potato hash diets. There is a need for further research on cost of producing fermented liquid potato hash and cost benefits of feeding fermented liquid potato hash.